HIV TREATMENT FAILURE MARKERS; VIROLOGIC OUTCOMES AFTER 12 MONTHS OF ANTIRETROVIRAL THERAPY AMONG PATIENTS RECEIVING HAART IN BUSIA COUNTY, KENYA

JAMES MUNYAO KINGOO

DOCTOR OF PHILOSOPHY

(Microbiology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2023

HIV Treatment Failure Markers; Virologic Outcomes after 12 Months of Antiretroviral Therapy among Patients Receiving HAART in Busia County, Kenya

James Munyao Kingoo

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in Microbiology of Jomo Kenyatta University of Agriculture and Technology

2023

DECLARATION

This thesis is my original work and has not been submitted for a degree in any other university.

Signature:... Date:..

James Munyao Kingoo

This thesis has been submitted for examination with our approval as university supervisors:

Signature:... Date:..

Prof. A. W. Muigai, PhD

JKUAT, Kenya

Signature:.. Date:..

Dr. Samoel A. Khamadi, PhD

KEMRI, Kenya

Signature:... Date:..

Prof. V. Matiru, PhD,

JKUAT, Kenya

DEDICATION

This work is dedicated to my dear wife, Philomena Mwikali who has always supported and motivated me to complete this work, my sons Tinashe Bunjwa and TimJames Kasyoka for their continuous motivation. I also dedicate this to the countless millions who have suffered at the hands of the AIDS epidemic over the years.

ACKNOWLEGDEMENTS

First and foremost, I would like to express my heartfelt thanks to my supervisors, Dr. Samoel Khamadi. Professor Anne Muigai and Professor Viviene Matiru. I greatly appreciate your meticulous guidance, patience, encouragement, leadership, moral support and the conducive environment that you created for me to complete my study smoothly.

I am extremely thankful to my dear wife Philomena Mwikali for her valuable support, encouragement, prayers and push towards completing this journey. My sons Tinashe and TimJames, having you in my life has been a great motivation towards making this happen.

I wish to convey my sincere thanks and acknowledgements to KEMRI – Centre of Virus Research team for their facilities and support during my research period. My gratitude goes to all the participants whose samples were used for this study.

Above all, I thank the Almighty God, my Enabler for giving me the inner strength and ability to accomplish this task. Indeed it has been the doing of the LORD; and it is marvellous in our eyes.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ACKNOWLEGDEMENTS iv

TABLE OF CONTENTS v

LIST OF TABLES xi

LIST OF FIGURES xiii

LIST OF APPENDICES xv

LIST OF ABBREVIATIONS xvi

ABSTRACT xxi

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background 1

1.2 HIV prevalence: A global Perspective 1

1.3 HIV prevalence in sub-Saharan Africa 2

1.4 HIV prevalence in Kenya 2

1.5 Previous studies on evaluation of markers of treatment failure 5

1.6 Statement of the problem 5

1.7 Justification 7
1.8 Research questions 7
1.9 Study objectives 8
1.9.1 General objective...8
1.9.2 Specific objectives...8
CHAPTER TWO..9
LITERATURE REVIEW 9
2.1 Origin and diversity of the Human Immunodeficiency Virus.............................9
2.2 HIV structure and genetics 10
2.3 Binding, entry and replication of HIV in the host cell 12
2.3 Binding, entry and replication of HIV in the host cell 13
2.4 HIV prevention and treatment...15
2.5 HIV treatment using antiretroviral therapy 16
2.6 Mechanism of action antiretroviral drugs...20
2.7 Development of HIV drug resistance against antiretroviral therapy..................22
2.8 Determination of HIV treatment failure in Kenya 25
2.9 HIV viral load testing among people living with HIV in Kenya 25
2.10 HIV drug resistance testing among people living with HIV in Kenya.............27

2.11 Combination of HIV viral load and drug resistance testing to determine treatment failure among people living with HIV in Kenya 27

CHAPTER THREE 29

MATERIALS AND METHODS 29

3.1 Study site 29

3.2 Study population 29

3.3 Study design 30

3.4 Inclusion criteria 30

3.5 Exclusion criteria 30

3.6 Sampling criteria and sample size determination 32

3.7 Ethical considerations 33

3.8 Benefits and risks to participants 34

3.9 Confidentiality of data and participant information 34

3.10 Laboratory testing procedures 35

3.10.1 Sample Preparation 35

3.10.2 HIV RNA quantification (viral load) testing 35

3.10.3 Extraction of HIV RNA for drug resistance testing 36

3.10.4 Reverse transcription of extracted HIV RNA for cDNA synthesis 37

3.10.5 Nested polymerase chain reaction 38

3.10.6 Analysis of PCR amplicons by gel electrophoresis 38

3.10.7 Sequencing of HIV positive specimen 39

3.10.8 Sequencing reactions and purifications 41

3.10.9 HIV sequence analysis 41

3.11 Statistical data analysis 44

CHAPTER FOUR 45

RESULTS 45

4.1 Participant demographic characteristics 45

4.2 HIV RNA quantitative (viral load) testing results among patients receiving HAART in Busia County, Kenya. 45

4.3 Drug resistance associated mutations (DRAMs) among patients receiving HAART in Busia County, Kenya. 48

4.3.1 NRTI DRAMs among patients receiving HAART in Busia County, Kenya. 51

4.3.2 NNRTI DRAMs among patients receiving HAART in Busia County, Kenya. 54

4.3.3 Protease inhibitors (PI) DRAMs among patients receiving HAART in Busia County, Kenya. 58

4.4 Drug resistance profiles among patients receiving HAART in Busia County, Kenya....59

4.5 HIV drug resistance scores among patients receiving HAART in Busia County, Kenya....62

4.6 HIV subtype variation among patients receiving HAART in Busia County, Kenya....63

4.7 Phylogenetic analysis among patients receiving HAART in Busia County, Kenya....67

CHAPTER FIVE 72

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 72

5.1 Discussion 72

5.1.1 HIV RNA viral load testing: a measure of HIV treatment failure among patients receiving HAART in Busia County, Kenya....73

5.1.2 HIV DRAMs conferring resistance to ART among patients receiving HAART in Busia County, Kenya 74

5.1.3 HIV subtype variation among patients receiving HAART in Busia County, Kenya....81

5.1.4 HIVDR resistance profiles, scores and phylogeny among patients receiving HAART in Busia County, Kenya 84

5.2 Conclusions 86

5.3 Limitations of the study....87

5.4 Recommendations 87

REFERENCES 89

APPENDICES 111

LIST OF TABLES

Table 3.1: A summary of the number of participants eligible to the study.....................32

Table 3.2: Primers used for polymerase chain reaction for the specific target gene region. 39

Table 4.1: Socio-demographic and clinical characteristics among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 46

Table 4.2: Number of NRTI, NNRTI and PI DRAMs among patients receiving highly active antiretroviral therapy in Busia County, Kenya................................50

Table 4.3: Characteristics of patients with APOBEC mutations among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 50

Table 4.4: A summary of Nucleoside Reverse Transcriptase Inhibitor (NRTI) HIV drug resistance associated mutations in patients receiving HAART in Busia, Kenya..52

Table 4.5: Summary of Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI) HIV drug resistance associated mutations in patients receiving highly active antiretroviral therapy in Busia, Kenya...55

Table 4.6: Summary of participants with E138 mutations among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 58

Table 4.7: Summary of Protease Inhibitor (PI) HIV drug resistance associated mutations in patients treated with highly active antiretroviral therapy in Busia, Kenya. 60

Table 4.8: Distribution of HIV subtypes circulating among patients receiving highly active antiretroviral therapy in Busia County, Kenya................................65

Table 4.9: Summary of demographic characteristics and DRAM profiles for sequences with CRFs among patients receiving highly active antiretroviral therapy in Busia, Kenya. 66

LIST OF FIGURES

Figure 2.1: Evolutionary relationships of human immunodeficiency virus type groups, subtypes, sub-subtypes, and recombinant forms 11

Figure 2.2: Schematic representation of HIV structure. 12

Figure 2.3: Schematic diagram of the HIV genome structure and organisation showing loci of the different genes. 13

Figure 2.4: Schematic representation of the four stages of infection risk and potential interventions. 16

Figure 3.1: Map of Busia Count, Kenya showing Busia County Referral Hospital, the sampling site. 31

Figure 3.2: Gel electrophoresis image following reverse transcription showing HIV-1 at 700bp, sample bands and molecular markers 40

Figure 4.1: Number of study participants with virologic failure at different age brackets among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 48

Figure 4.2: Number of NNRTI, NRTI and PI DRAMs among patients receiving highly active antiretroviral therapy in Busia County, Kenya grouped by age bracket. 51

Figure 4.3: Number of resistance profiles for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. .. 53

Figure 4.4: Number of phenotypic resistance profiles for different NNRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 57

Figure 4.5: Distribution of the drug resistance profiles for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 61

Figure 4.6: Distribution of the phenotypic drug resistance profiles for different NNRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 62

Figure 4.7: Distribution of different DRAMs among subtypes for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 67

Figure 4.8: Distribution of subtypes among male and female participants for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 68

Figure 4.9: Phylogenetic tree of the HIV pol-RT gene showing HIV subtype A1 and HIV-1 subtype A2 among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 69

Figure 4.10: Circular phylogenetic tree of the HIV pol-RT gene among patients receiving highly active antiretroviral therapy in Busia County, Kenya. .. 70

Figure 4.11: Phylogenetic tree of the HIV pol-RT gene showing HIV subtype A, HIV subtype D, HIV subtype A1_D, HIV subtype A1_C, HIV subtype G and HIV subtype C among patients receiving highly active antiretroviral therapy in Busia County, Kenya. 71

LIST OF APPENDICES

Appendix I: Stanford University HIV Drug Resistance Database Generated Clinical Report 111

Appendix II: GenBank Accession Numbers 116

Appendix III: Ethical Approval 122

Appendix IV: Study Informed Consent Form. 123

LIST OF ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome.
AGYW	Adolescent Girls and Young Women
ART	Antiretroviral Therapy
AZT	Azidothymidine
BLAST	Basic Local Alignment Search Tool
CA	Capsid
CCC	Comprehensive Care Centre
CCR5	CC chemokine receptor 5
CD4	helper T cells
CRF	Circulating Recombinant Forms
CXCR4	C-X-C Chemokine Receptor 4
DD1	Didanosine
DNA	Deoxyribonucleic Acid
DPS-FP	Dried plasma spots on filter paper
DPS	Dried plasma spot
DR	Drug Resistance

DRAMs	Drug Resistance Associated Mutations
DRT	Drug Resistance Testing
EDTA	Ethylenediaminetetraacetic acid
EFV	Efavirenz
ENV	*Viral envelope*
FUCHIA	Follow-up and care of HIV Infection and AIDS, Epicentre, Paris
FP	Filter Paper
GAG	*Group Antigens*
GP120	*Glycoprotein 120*
GP41	*Glycoprotein 41*
HAART	High Active Antiretroviral Treatment
HIV	Human Immune Deficiency Virus
HIV-1	Human Immune Deficiency Virus Type 1
HIV-2	Human Immune Deficiency Virus Type 2
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
ID	Identifier
IN	*Integrase*
KAIS	Kenya AIDS Indicator Survey

KEMRI Kenya Medical Research Institute

MA Matrix

MEGA Molecular Evolutionary Genetics Analysis

Ml Milliliter

MOH Ministry of Health

MSF Médecins Sans Frontieres

NASCOP National AIDS/STI Control Program

NEF Negative Regulating Factor

NC Nucleocapsid

NJ Neighbour Joining

NLV Nelfinavir

NRTI Nucleoside Reverse Transcriptase Inhibitor

NNRTI Non-nucleoside Reverse Transcriptase Inhibitor

NVP Nevirapine

OI Opportunistic Infections

PC Personal Computer

PI Protease Inhibitor

PLWHA People Living With HIV and AIDS

PMTCT Prevention of Mother to Child Transmission

POL *Polymerase*

PPT Polypropylene Tube

PR *Protease*

REV *Amino Acid Nuclear Regulatory Protein Gene*

RNA Ribonucleic Acid

RT *Reverse Transcriptase*

RT Reverse Transcription

3TC Lamivudine

TAT *Transactivator of Transcription*

VCT Voluntary Counselling Testing

VIF *Viral Infectivity Factor*

VL Viral Load

VPR *Virus Protein R*

VPU *Virus Protein Unique*

UNAIDS Joint United Nations Programmes on HIV/AIDS

UNICEF United Nations Children Educational Fund

URF Unique Recombinant Forms

UVL Undetectable Viral Load

VS Viral Success

WHO World Health Organization

ABSTRACT

Data on virologic outcomes and the development of Human Immunodeficiency Virus (HIV) drug resistance in patients receiving antiretroviral therapy (ART) in resource-limited programmatic settings in sub-Saharan Africa is scanty. Thus, the need to describe the emergence of virologic failure and ART resistance patterns among patients treated with the standard highly active antiretroviral therapy regimens in these settings. The Kenyan Ministry of Health while collaborating with other stakeholders in the HIV field has been treating HIV infected patients in Busia County Referral Hospital, Western Kenya, since July 2003. While viral load testing as a marker of treatment failure is now routinely performed on patients on ART, HIV drug resistance testing (DRT) is not performed as a standard of care in majority of the testing facilities owing to the high cost and technical skill required to perform the test. The main objective of this cross-sectional study was to assess the effectiveness of ART in terms of virologic outcomes among patients receiving HAART for 12 months or more in Busia County Referral Hospital, Western Kenya. Blood samples from patients receiving first line therapy for >12 months at the hospital's comprehensive care centre were collected and viral load testing performed. Samples with virologic failure (>1000 copies/ml) were genotyped to determine HIV drug resistance associated mutations (DRAMs) in the protease and *reverse transcriptase (RT)* regions of the HIV genome and HIV subtypes. Phylogenetic analysis was carried out using Molecular Evolutionary Genetics Analysis (MEGA) phylogenetic software version 11.0. The results showed a virologic failure of 15.9% (146/915 participants). Subtype A1 was the most predominant subtype (52.3%). Eighty-seven (62%) of participants with virologic failure had at least one major HIV DRAM against either the protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or a combination of two or more of the above and exhibited resistance against one or more antiretroviral drugs. Of these 87 participants with at least one DRAM, 65 (74.7%) were females while 22 (25.3%) were males. A total of 393 DRAMs against PIs, NNRTIs and NRTIs were identified in the study population as follows: PI DRAMs = 12, NNRTI DRAMs = 197 and NRTI DRAMs = 184. K103N/S was the most prevalent NNRTI DRAM while M184V was the predominant NRTI DRAM. Phenotypically, 39.3% of participants with virologic failure exhibited susceptibility to all tested ART drug classes. After phylogenetic analysis, the different subtypes clustered with reference sequences from around the globe with inter and intra-subtype cluster differences. This study concludes that HIV viral RNA quantification (viral load testing) may be used as a predictor of treatment failure in HIV seropositive patients receiving ART with 62.1% of those with virologic failure having one or more DRAMs that conferred resistance to one or more classes of drugs tested. The most predominant subtype circulating in the population is A1, with increased subtype D circulating recombinant forms (CRF) prevalence. Combination of viral load and drug resistance testing will find utility in better predicting treatment failure and assisting in making a decision on regimen change. This study recommends continued monitoring of the circulating subtypes, especially with the reported increase

in CRFs and the mutation/resistance patterns to help predict treatment failure patterns, transmission rates, rates of recombination and disease progression within populations. Since not every treatment failure is associated with viral mutations, drug resistance testing should be provided to rule out adherence related treatment failure before any regimen switch is effected.

CHAPTER ONE

INTRODUCTION

1.1 Background

Treatment of human immunodeficiency virus (HIV) using antiretroviral therapy (ART) drugs is among the greatest developments in the management of HIV infection since the early 1980s when HIV was isolated as the etiologic agent of acquired immunodeficiency syndrome (AIDS) (Zulfiqar *et al.,* 2017). In the late 1980's to mid-1990's, there were limited options for HIV treatment with the clinical management consisting mainly the management of common opportunistic infections and AIDS related illnesses. The initial HIV treatment in the late 1980's to early 1990's was given as a monotherapy (Kemnic & Gulick, 2019), with a steady evolution to combination therapy that has seen a cocktail of HIV medications being used to attain its clinical management from mid-1990's to date (Kemnic &Gulick, 2019; Tseng *et al.,* 2015).

1.2 HIV prevalence: A global Perspective

As of end of 2010, approximately 35 million people were reported to be living with HIV globally, an increase of 17% from 2001 (del Rio, 2017; Lenjiso *et al.,* 2019; Sonali *et al.,* 2019). The estimates increased in 2017 to about 36.7 million PLWH worldwide, with close to 30 million (~81.7%) of these living in low- and middle-income countries (LMICs) mainly Sub-Saharan Africa, depicting skewed prevalence of HIV between the developed and developing world (Günthard *et al.,* 2016; Joint United Nations Programme on HIV/AIDS, 2018a, 2018d; Kharsany & Karim, 2016). Before 2019 came to a close, there were more than 38 million PLWH globally (Joint United Nations Programme on HIV/AIDS, 2020a). Global preliminary findings indicate that approximately 37.7 million people were living with HIV in 2020 with 1.5 million new infections and 680,000 AIDS-related deaths (Joint United Nations Programme on HIV/AIDS, 2020, 2021; Vardell,2020). The increase in both HIV incidence and AIDS related mortality and morbidity has stalled over the years, reflecting the benefits of

improved access to ART (de Cock *et al.,* 2021; Günthard *et al.,* 2016; World Health Organization, 2021). About 21.7 million people living with HIV in 2017 had access to antiretroviral therapy (Joint United Nations Programme on HIV/AIDS, 2018a, 2018c, 2021; World Health Organization, 2017), a figure that steadily increased to 28.7 million people by June 2021 (Joint United Nations Programme on HIV/AIDS, 2021b).

1.3 HIV prevalence in sub-Saharan Africa

Majority of the global HIV cases are found in sub-Saharan African region which bears at least 76% of the total global infections (Kharsany *et al.,* 2019; Kharsany & Karim, 2016), 76% of the global new HIV infections, and 75% of the total reported AIDS related deaths (Joint United Nations Programme on HIV/AIDS, 2018d ; Kharsany *et al.,* 2019; Kharsany & Karim, 2016). The situation is compounded by the fact that most sub-Saharan African countries are low and medium income economies with limited budgets on health (Kharsany *et al.,* 2019; Kharsany & Karim, 2016). Despite increasing ART coverage in the region to-date, sub-Saharan Africa still contributes to the highest number of AIDS related deaths globally (Kharsany *et al.,* 2019; Kharsany & Karim, 2016; Vardell, 2020). Official estimates suggest that 6,000 new HIV infections are recorded daily across the globe (Kharsany & Karim, 2016), and two thirds of these are in sub-Saharan Africa (Govender *et al.,* 2021; Kharsany & Karim, 2016), where women and young girls bear the biggest burden, with adolescent girls and young women aged between 15-24 years having almost eight times higher probability of HIV infection than males (Govender *et al.,* 2021; Kharsany & Karim, 2016). Recent data shows that adolescent girls and young women (AGYW) between the ages of 15-24 years in sub-Saharan Africa are increasingly at a higher risk of HIV with AGYW accounting for 63% of all new HIV infections in 2021 alone (Govender *et al.,* 2021).

1.4 HIV prevalence in Kenya

HIV prevalence in Kenya was estimated 5.9%, translating to 1.5 million Kenyans living with HIV in 2015 with annual new HIV infections in Kenya estimated to be 77,647 in

2015. (National AIDS and STI Control Programme [NASCOP] 2016, 2018; National AIDS Control Council KENYA, 2016). Thea prevalence further reduced to 4.9% by 2017 (National AIDS Control Council, 2018; National AIDS and STI Control Programme (NASCOP), 2018; Onyango *et al.,* 2021). Over the years, a significant decrease in the number of people living with HIV (both adults and children) in Kenya with the overall HIV prevalence among Kenyans of ages 15-49 years dropping from 7.1% in 2007 (Onyango *et al.,* 2021) to 5.6% in 2012 (Kimanga *et al.,* 2014), down to 4.9% in 2019 (National AIDS and STI Control Programme (NASCOP), 2018; Onyango *et al.,* 2021) and finally down to 4.2% in 2020 (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2020b; Joint United Nations Programmes on HIV/AIDS (UNAIDS), 2018).

Antiretroviral coverage greatly increased to 75% and 82% for adults and children respectively in 2017 (National AIDS Control Council, 2018) up from 66% in 2015 (National AIDS Control Council KENYA, 2016), resulting in reduced mortality, lower transmission rates and improved lives due to lower morbidity (Joint United Nations Programmes on HIV/AIDS, 2018; National AIDS Control Council, 2018). Antiretroviral therapy provision in Kenya increased from less than 10,000 people in 2003 to over 430,000 people in December 2010 mainly as a the rapid acceleration and expansion of HIV treatment, care and management services (Joint United Nations Programmes on HIV/AIDS, 2011). As of early 2009, estimated 190,000 HIV-infected Kenyans received ART, which was only 44% of those in need of HIV treatment in Kenya at the time (National AIDS and STI Control Programme (NASCOP), 2018). By the end of 2015, it was estimated that close to 900,000 people out of the total 1.6 million HIV infected people in Kenya were under ART treatment (representing ~56% coverage), a figure that crossed the 1 million mark in 2016 (National AIDS Control Council Kenya, 2016). ART treatment coverage in Kenya increased to 75% in 2019 as shown in **Figure** 1.1 (Joint United Nations Programmes on HIV/AIDS, 2018c). Generally, Kenya has performed better than many high burden countries in increasing percentage ART

coverage, even surpassing some developed countries (Joint United Nations Programmes on HIV/AIDS, 2018d).

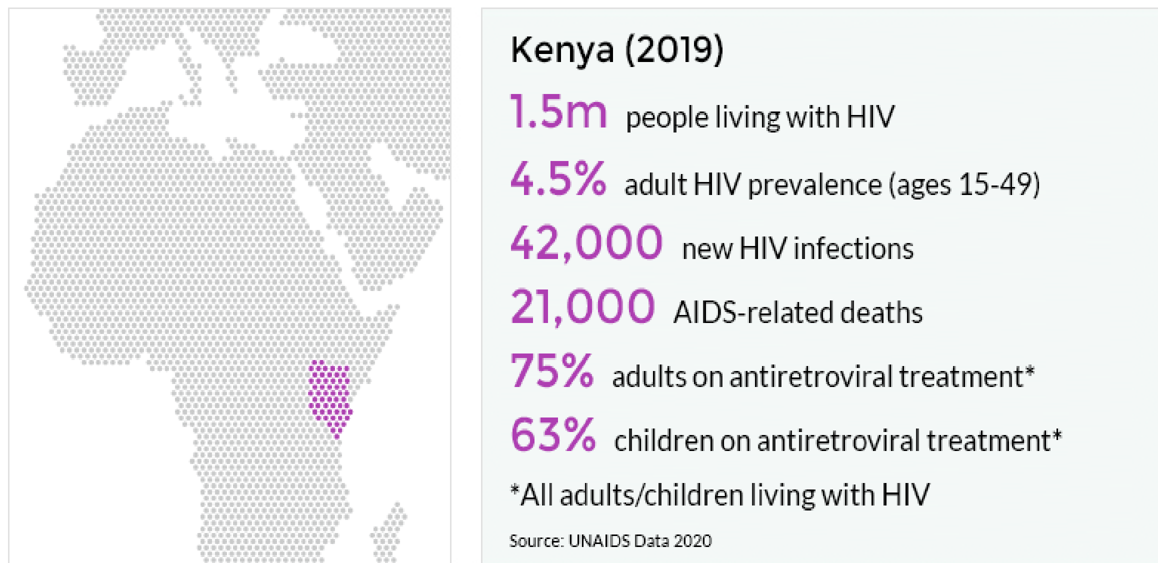


Figure 1.1: Kenyan HIV statistics 2019 (Joint United Nations Programme on HIV/AIDS (UNAIDS) Data 2020).

Kenya is among the countries with the highest HIV burden globally: registering the fourth largest epidemic globally (Kantor *et al.,* 2014; Kassaye *et al.,* 2019; Kimanga *et al.,* 2014), with an estimated 1.5 million adults reported to be living with HIV by 2018, an alarming 90,000 news infections on average every year hence a prevalence of ~4.9% (National AIDS Control Council, 2018). As a result, Kenya has made a commitment to the World Health Organization's HIV treatment strategy to diagnose 90% of all HIV infected people, put 90% of those who test positive on ART and ensure that 90% of those on ART treatment are virally suppressed (commonly referred to as the 90-90-90 treatment strategy) by the year 2020 hence reducing the HIV pandemic to a mere endemic disease by 2030 (Cherutich *et al.,* 2016). Kenya is also a signatory of the revised 95-95-95 HIV targets by the year 2030. This subpopulation of ~24% patients in this study with detectable viremia despite being on medication for over 12 months

means that extra efforts are required to bring down treatment failure for the 95-95-95 strategy to be actualized.

1.5 Previous studies on evaluation of markers of treatment failure

Various studies using similar treatment regimens and either serum, dried plasma spots/dried blood spots (DPS/DBS) or plasma as the sample type, have assessed the level of virologic suppression achieved under routine conditions in resource-poor settings. Viral suppression after 6 months of treatment ranged from between 63-66% in Thailand and Uganda to more than 80% in Botswana and South Africa (Bessong *et al.,* 2021). Cherutich *et al*. (2016), in their analysis of the Kenya AIDS Indicator Survey (KAIS) 2012 data reported that overall, 61.2% of HIV infected Kenyans aged between 15-64 years had not achieved viral suppression and 26.1% of those on ART had viral loads above the minimum level of detection at the start of the survey. In addition, the utilization of dried plasma and/or dried serum spots for serologic or molecular diagnosis of HIV infection and molecular epidemiology of HIV diversity has been scrutinized widely (Levine *et al.,* 2016; Tuaillon *et al.,* 2020). The fact that dried spots are easy to prepare, store and transport potentially makes this an important individual and epidemiological HIV monitoring tool (Levine *et al.,* 2016; Tuaillon *et al.,* 2020). A study by Kantor *et al*. (2014) reported 1.8% resistance in ART naïve patients, and over 91% treatment failure through drug resistance testing for ART experienced patients in Western Kenya. Additionally, several studies have also reported varying degrees of drug resistance associated mutations for plasma, serum, DBS, DPS, ViveST™-plasma as well as ViveST-blood, with plasma recording a higher number of mutations compared to the rest (Levine *et al.,* 2016; Tuaillon *et al.,* 2020).

1.6 Statement of the problem

The Ministry of Health in Kenya has expanded the provision of antiretroviral therapy to all HIV-positive individuals since 2003 (Wekesa *et al.,* 2020; National AIDS Control Council Kenya, 2016; US Presidential Emergency Plan for AIDS Relief, 2018).

Currently, there is limited access of HIV drug resistance testing (DRT) among people initiated to ART in programmatic resource limited settings in Kenya and other Sub-Saharan African countries bearing the highest HIV burden (Moyo *et al.,* 2020). Several studies have described patterns of HIV resistance associated mutations in treatment naïve patients as well as patients on ART in resource limited settings. These results suggest that settings where patients are first treated with highly active antitretroviral therapy (HAART) may demonstrate lower rates of drug resistance development as compared to those initiated on other first line therapies. With increasing numbers of patients that are being initiated to ART and the rapid scale up of ART in Kenya, HIV DRT is recommended prior to initiation of ART for better clinical outcomes.

Little is known on HIV drug resistance (DR) patterns in resource limited settings like Busia County, Kenya. Mapping of drug resistance patterns as well is critical for formulation of policies towards HIV management and treatment. Although viral load is now a standard of care in determining treatment failure, data on combination of viral load and HIV DRT to improve the management of people living with HIV within Busia County is scanty. This poses challenges to the testing programme especially when there is need to switch medication due to treatment failure. Few studies have been carried out to determine the circulating HIV subtypes and their phylogenetic relationships within Busia County. Lack of adequate data on the circulating HIV subtypes causes challenges in management of people living with HIV within Busia County since the type and combinations of circulating subtypes will have impacts on management and treatment of patients. Until sufficient data on the phylogenetic relationships between the circulating HIV subtypes within Busia County and evolutionary patterns, it will be challenging to map the directions from which the HIV epidemic in Kenya is fueled which is important in formulating preventive policies and strategies. Additionally, Busia county is among the five highest HIV-1 burdened counties in Kenya, with a prevalence of 7.7%, a figure higher than the overall national prevalence of 4.9% (Makwaga *et al.,* 2020).

1.7 Justification

The necessity to continually evaluate the emergence of HIV drug resistance among patients on ART is long overdue especially in resource limited settings of Sub-Saharan Africa where majority of the global HIV infections and AIDS related deaths occur. There is need to describe viral load and DR patterns in resource constraint settings like in Busia, Kenya, to help policy makers in developing policies for DRT programs in within Busia County and Kenya. Determination of circulating HIV subtypes within Busia County is important for better understanding of disease epidemiology, progression and response to ART administration. More so, evaluation of DR patterns and circulating HIV strains in Busia so as to rule out any possibilities of cross border infections is important since Busia is a border town which is important in formulating policies against cross-border infections. Phylogenetic analysis will assist in determining the different directions from which the HIV epidemic in Busia County and Kenya as a whole which is important in formulating cross border prevention policies and strategies. This study contributes to the discussion on whether viral load testing alone is sufficient to monitor HIV treatment failure in resource-limited settings or whether HIV DRT should be incorporated to improve treatment outcomes. Therefore, this study aimed at describing DR patterns in this drug experienced population of Busia County. The study site was selected due to the existence of a well-established HIV treatment centre, the high HIV prevalence in the area and its proximity to the border with Uganda, where the circulating HIV subtype profiles are different from the ones recorded in Kenya. The results from this study will lead to formulation of HIV management and prevention strategies which could be applicable to other region of Kenya and globally in other resource limited settings.

1.8 Research questions

1. What are the levels and patterns of HIV drug resistance associated mutations (DRAMs) among patients showing virologic failure in Busia County, Kenya?

2. What is the role of viral load and HIV drug resistance testing in improving antiretroviral therapeutic approach of patients within Busia County, Kenya?
3. What are the circulating HIV subtypes in Busia County, Kenya?
4. What are the phylogenetic differences between the various virus strains circulating in Busia County, Kenya?

1.9 Study objectives

1.9.1 General objective

To evaluate treatment failure markers; virological outcomes after 12 months of standard antiretroviral therapy in Busia County, Kenya.

1.9.2 Specific objectives

1. To determine how viral load and HIV drug resistance testing may improve the management and antiretroviral therapeutic approach among HIV positive patients in Busia County, Kenya.
2. To determine the level of HIV drug resistance mutation patterns among HIV positive patients not achieving virologic suppression in Busia County, Kenya.
3. To determine the HIV subtypes circulating in Busia County, Western Kenya.
4. To determine the phylogenetic distances between different HIV strains circulating in a cross-sectional study in Busia County, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and diversity of the Human Immunodeficiency Virus

The Human Immunodeficiency Virus (HIV) is a retrovirus grouped to the genus Lentivirus within the Retroviridae family, subfamily Orthoretrovirinae (Seitz, 2016). Retroviruses contain an outer glycoprotein envelope and an internal core that consists of two identical single stranded RNA molecules and three enzymes; RNA transcriptase, integrase and protease that are critical for viral replication (Seitz, 2016; Vaillant & Gulick., 2020). HIV is classified into two types: HIV type 1 (denoted as HIV-1) and HIV type 2 [denoted as HIV-2] (Gomes *et al.,* 2020; Tebit *et al.,* 2016). HIV-1 is believed to have originated from the chimpanzee simian immunodeficiency viruses (SIVs), which infected several geographically isolated chimpanzee communities in Cameroon (Gomes *et al.,* 2020). Both HIV-1 and HIV-2 have been traced to multiple cross-species transmissions with SIVs from chimpanzees, sooty mangabeys and western gorillas (Faria *et al.,* 2019; Gomes *et al.,* 2020). There are speculations that both HIV-1 and HIV-2 afterwards spread among humans from Cameroon along the Congo River (because of enhanced transportation down the river) into Kinshasa (Faria *et al.,* 2019), Democratic Republic of Congo (formerly known as Zaire), where the earliest confirmed case of HIV infection (with group M strain) in humans was traced to a stored blood sample collected from a patient in 1959 (Faria *et al.,* 2019).

HIV-2 is a rare type only localized to some parts of West Africa, although an increasing prevalence has been reported in other parts of the globe, including Europe and the Unites States of America (Gomes *et al.,* 2020). HIV-2 infection in individuals can take up to thirty years to show the first signs of AIDS (Gomes *et al.,* 2020). HIV-1 (referred to as HIV in the rest of this document) contributes to most cases of HIV infections globally (Rubio-Garrido *et al.,* 2020). HIV can be classified into four groups: the "major" group M, the "outlier" group O, and the "new" group N and the recently discovered group P

(Rubio-Garrido *et al.,* 2020), as a result of variations of the genome in the virus' *env* region (Rubio-Garrido *et al.,* 2020; Seitz, 2016; Tough & McLaren, 2019). The HIV groups M, N, O and P represent different pathways through which SIVs were introduced into humans (Désiré *et al.,* 2018; Tough & McLaren, 2019). While SIV strains related to HIV groups M and N have been identified in chimpanzees, there is sufficient evidence suggesting that both HIV groups O and P originated from gorillas, especially Western gorillas (Tough & McLaren, 2019), in which the closest SIV relatives of this group have been described (Tough & McLaren, 2019). Within the HIV group M, there are known to be at least nine genetically distinct subtypes (or clades) and there are at least ten confirmed genetically distinct subtypes of HIV namely subtypes A, B, C, D, F, G, H, J, K and L as shown in Figure 2.1 (Rubio-Garrido *et al.,* 2020).

Sometimes, two or more HIV viruses of different subtypes inside the cell of an individual can combine their genetic material to create a new hybrid virus resulting in inter-subtype recombinants (Reis *et al.,* 2019; Recordon-Pinson *et al.,* 2018). When the HIV recombinants are transmitted and spread within a population, they are recognized classified as circulating recombinant forms [CRFs] (Tongo *et al.,* 2016) whereas HIV unique recombinant forms (URFs), are the recombinants that have been sampled only once from a single multiply-infected individual (Reis *et al.,* 2019; Tongo *et al.,* 2016). It is important to note that in East Africa, where HIV subtypes A, D and C predominate, CRF AD and CRF AC are most common (Giovanetti *et al.,* 2020).

2.2 HIV structure and genetics

The HIV particle is surrounded by a bi-lipid layer known as the viral envelope or membrane (Huarte et al., 2016; Seitz, 2016). Projecting from the HIV viral envelope are 72 spike-like structures, formed from the glycoproteins gp120 and gp41 (Huarte et al., 2016). The total number of the spikes (gp120/gp41 trimers) per virus which is dependent on the HIV isolate ranges between 10 and 100 (Giovanetti et al., 2020; Huarte et al., 2016).

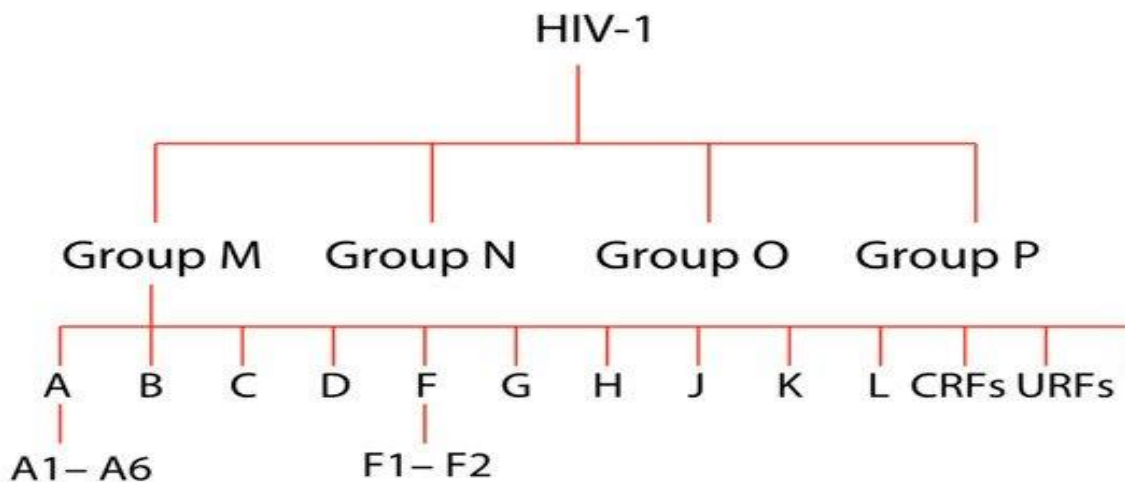


Figure 2.1: Evolutionary relationships of human immunodeficiency virus type groups, subtypes, sub-subtypes, and recombinant forms (Giovanetti et al., 2020).

Underneath the viral envelope is the viral matrix, which is made from the protein *p17* (Giovanetti *et al.,* 2020; Seitz, 2016). The viral core (or capsid) is bullet-shaped and is composed of the protein *p24* (Huarte *et al.,* 2016; Seitz, 2016). Inside the HIV core are the reverse transcriptase, integrase and protease (Figure 2.2); the three enzymes required for HIV replication (Seitz, 2016; Tian *et al.,* 2018). Also resident within the core is HIV's genetic material, composed of two identical RNA strands (Huarte *et al.,* 2016; Seitz, 2016; Tian *et al.,* 2018). The *RT* is an enzyme containing two enzymatic activities; a Deoxyribonucleic Acid (DNA) polymerase activity that is able to copy both RNA and DNA templates, and an RNase H activity which degrades RNA within a RNA/DNA duplex (Huarte *et al.,* 2016; Tian *et al.,* 2018). RT uses these two enzymatic activities to reverse transcribe the single-stranded RNA to a double-stranded DNA in preparation for integration into the host cell genome (Giovanetti *et al.,* 2020; Tian *et al.,* 2018).

HIV genes are located in the central core of the pro-viral DNA and are involved in the coding of at least nine functional proteins (Bbosa *et al.,* 2019; Seitz, 2016). These proteins are divided into three classes namely: The major structural proteins (*Gag, Pol,*

and *Env*), regulatory proteins (*Tat* and *Rev*), and accessory proteins which include *Vpu, Vpr, Vif,* and *Nef* (Bbosa *et al.,* 2019; Seitz, 2016; Zulfiqar *et al.,* 2017).

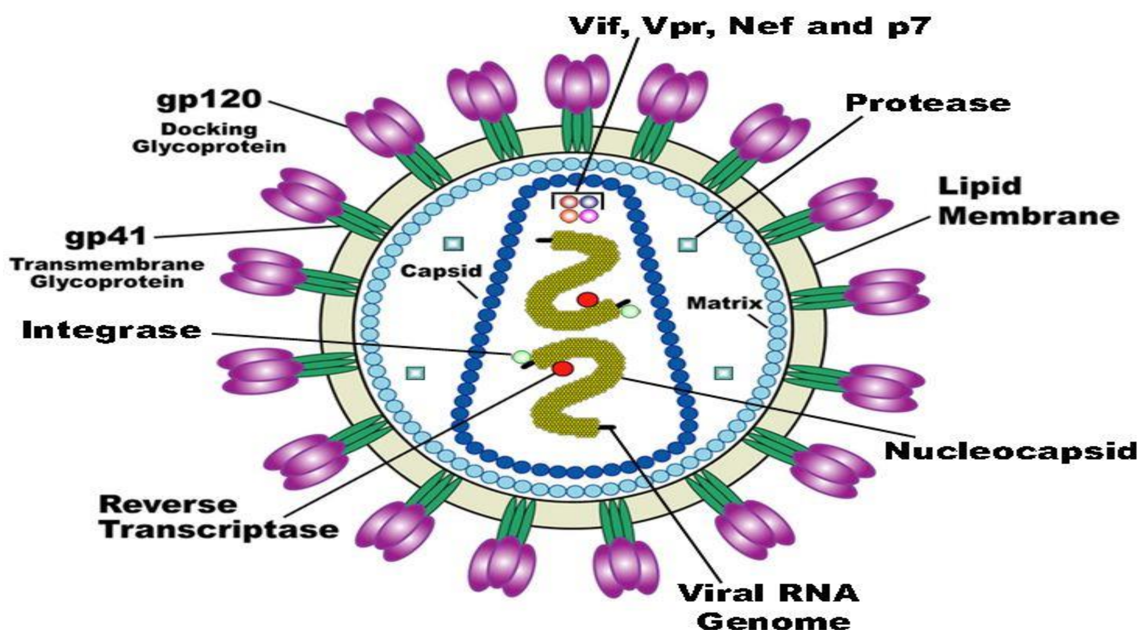


Figure 2.2: Schematic representation of HIV structure (Engelman & Cherepanov, 2012)**.**

The entire genome of HIV consists of two single-stranded RNA molecules enclosed within the capsid core of the virus as indicated in Figure 2.3 (Bbosa *et al.,* 2019; Seitz, 2016), and is primarily a coding RNA containing nine open reading frames which produce 15 important viral proteins (Seitz, 2016). Of the nine open reading frames encoded by the HIV genome, three encode the *Gag, Pol*, and *Env* polyproteins, which are later proteolyzed into individual proteins which are common to all retroviruses (Bbosa *et al.,* 2019).

2.3 Binding, entry and replication of HIV in the host cell

The transmission of HIV is dependent on biological properties of viral strains, and individual susceptibility (Seitz, 2016). Binding to a receptor on the surface of the host

cell (a CD4 antigen on helper T-cells, monocyte/macrophages, dendritic or glial cells), is an initial mandatory requirement before the virus infects the cell (Seitz, 2016; Woodham *et al.,* 2016). Entry of HIV into cells is mediated by interaction between CD4 cells and chemokine receptors that serve as entry co-receptors (Smith *et al.,* 2021; Woodham *et al.,* 2016). Woodham *et al*. (2016), have also demonstrated that HIV utilizes the CD4 receptor found on some cells of the immune system and the chemokine binding co-receptor to gain entry into human cells.

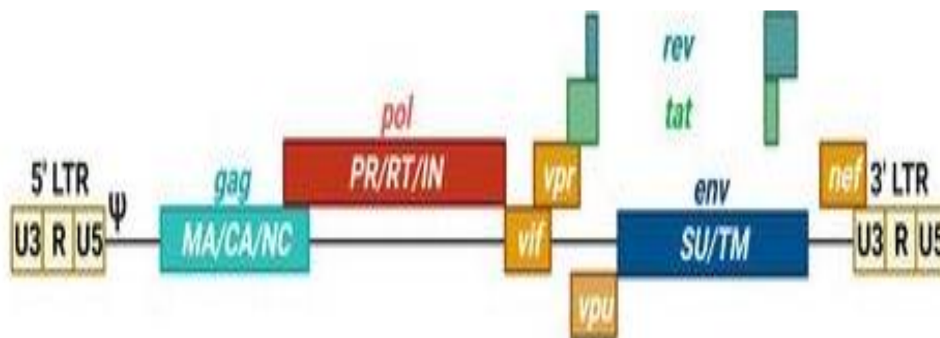


Figure 2.3: Schematic diagram of the HIV genome structure and organisation showing loci of the different genes (van Heuvel & Schatz, 2022).

2.4 Binding, entry and replication of HIV in the host cell

The transmission of HIV is dependent on biological properties of viral strains, and individual susceptibility (Seitz, 2016). Binding to a receptor on the surface of the host cell (a CD4 antigen on helper T-cells, monocyte/macrophages, dendritic or glial cells), is an initial mandatory requirement before the virus infects the cell (Seitz, 2016; Woodham *et al.,* 2016). Entry of HIV into cells is mediated by interaction between CD4 cells and chemokine receptors that serve as entry co-receptors (Smith *et al.,* 2021; Woodham *et al.,* 2016). Woodham *et al*. (2016), have also demonstrated that HIV utilizes the CD4 receptor found on some cells of the immune system and the chemokine binding co-receptor to gain entry into human cells. The entry of HIV into brain cells is initiated by a

high-affinity interaction between *gp*120 and CD4 which induces a conformational change in *gp120* that exposes the binding site for either CCR5 or CXCR4 chemokine co-receptor for attachment (Smith *et al.,* 2021). The glycoprotein *gp120* on the surface of the HIV particle binds to CD4 cells during infection and the co-receptor forming the virus-host cell co-receptor complex which allows the viral envelope to fuse with the host cell membrane which facilitates the viral entry into the host cell (Seitz, 2016).

Following successful entry into the cell, the HIV virus replicates by multiplication of the viral genomic material, proteins and subsequent reconstitution of new viruses (Seitz, 2016). HIV RNA is reverse transcribed into double stranded cDNA called the provirus by the action of the enzyme reverse transcriptase (Coffin *et al.,* 2021; Seitz, 2016). Reverse transcriptase has been identified as the major target for most of the antiretroviral drugs against HIV globally (Günthard *et al.,* 2016). Viral integration then takes place where the newly synthesized provirus DNA strand is incorporated into the host cell DNA with the help of the integrase enzyme (Coffin *et al.,* 2021; Seitz, 2016). Integration of HIV pro-viral DNA into a host cell chromosome is a critical step in the replication cycle of HIV. Once integrated, the pro-viral DNA is replicated along with the host cellular DNA during cycles of cell division (Ciuffi, 2016; Coffin *et al.,* 2021). The provirus serves as the template from which viral RNAs are transcribed (Coffin *et al.,* 2021; Ciuffi, 2016). The integrated provirus then transcribes the messenger RNA (mRNA) which is then translated to new viral proteins used in the reconstitution of new viral particles (Coffin *et al.,* 2021).

As the virion assembles itself, it packages all components required for infection of host cells (Zulfiqar *et al.,* 2017). These components include two copies viral RNA, cellular tRNALys, the viral envelope (*Env*) protein, the *Gag* polyproteins, and the three viral enzymes: protease (*PR*), reverse transcriptase (*RT*), and integrase (*IN*) required for proliferation (Freed, 2015). These newly formed viral particles then bud off the host cell to attack other cells (Coffin *et al.,* 2021; Ciuffi, 2016; Freed, 2015).

2.5 HIV prevention and treatment

To date, more than thirty-five years into the HIV epidemic and the discovery of HIV as the etiology agent of AIDS, there is no known cure for AIDS (de Cock *et al.,* 2021; World Health Organization, 2017). In the mid-1990s, the development and introduction of combination therapy that reduced viral load levels in HIV infected individuals generated optimism for HIV cure in form of a vaccine (Ng'uni *et al.,* 2020; Tseng *et al.,* 2015). The vaccine was rendered ineffective after the discovery of resting T cells with integrated HIV viral genomes that could not produce infectious virus while in a resting state but could do so upon T cell activation (Deeks *et al.,* 2015; Ng'uni *et al.,* 2020). Over time, considerable efforts have been put towards a search for HIV cure, especially a functional vaccine without much success (Ng'uni *et al.,* 2020).

A combination of several prevention strategies has been employed with a great degree of success to reverse the high prevalence and incidence rates as well as to slow down HIV transmission rates (Kharsany & Karim, 2016; Tseng *et al.,* 2015). Several HIV prevention interventions such as awareness of one's HIV sero-status, abstinence, behavior change to reduce risky behavioral patterns, use of condoms, voluntary male circumcision (Kharsany & Karim, 2016), discouraging exchange of needles especially for injecting drug users, timely diagnosis and treatment of sexually transmitted infections, as well as the use of medications by both HIV-infected (ART) and uninfected but exposed persons (PEP and PrEP) [Figure 2.4] have shown potency in partially protecting against HIV transmission and acquisition (Kharsany *et al.,* 2019).

Using ART for HIV treatment (commonly known as treatment for prevention) has shown high efficacy in preventing transmission of the virus in several studies and clinical trials (Cohen *et al.,* 2016; Kharsany & Karim, 2016). There is sufficient evidence that the provision of an effective ART against HIV-infected individuals reduces sexual transmission from a sero-positive partner to a sero-negative partner in a discordant relationship by 96% (Cohen *et al.,* 2016; Kharsany & Karim, 2016).

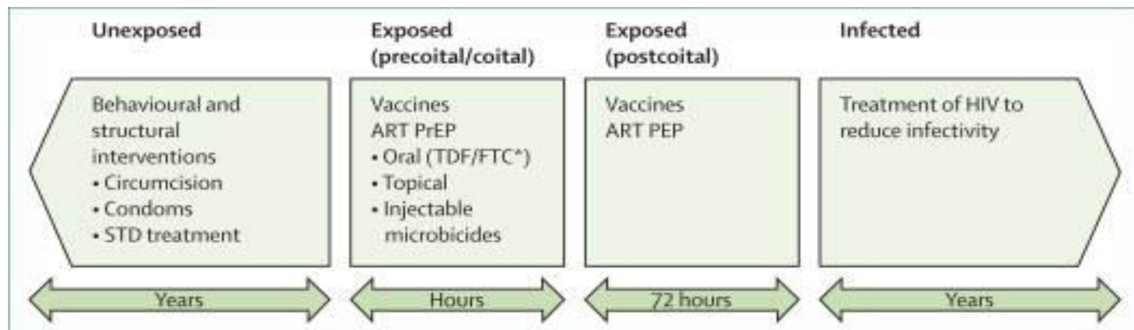


Figure 2.4: Schematic representation of the four stages of infection risk and potential interventions (Cohen *et al*. 2016).

2.6 HIV treatment using antiretroviral therapy

In the early 1990's, prophylaxis against common opportunistic infections such as pneumonia, Salmonella infection, candidiasis, toxoplasmosis, pneumonia and tuberculosis (TB) among others was the main form of clinical management of HIV (Ganapathy & Mozhi, 2021). Management of AIDS-related illnesses in the 90s was the major form of HIV management since few antiretroviral treatment options for treatment of HIV infection existed especially in sub-Saharan Africa (Ganapathy & Mozhi, 2021). The development of inhibitors of the RT and PR enzymes and the introduction of combination therapies to enhance the overall efficacy and durability of therapy revolutionized the treatment of HIV-1 infection in the mid 1990's (Günthard *et al.,* 2016; Tseng *et al.,* 2015).

This combination therapy used as treatment against HIV is now commonly referred to as ART which has led to decrease in the number of deaths and the rate at which people disease progresses in HIV infected people since its roll out in 1996 (Kemnic & Gulick, 2019). Currently, there are more than 25 drugs licensed for clinical use in HIV treatment, a previously well-known non-treatable and not easily managed ailment (Kemnic &Gulick, 2019). The syndrome has been reduced to a chronic disease which is

now easily manageable thanks to the development of these anti-HIV drugs (Sonali *et al.,* 2019). The key goals of ART include: to ensure the achievement and maintenance of suppression of plasma viremia to below the detection level of the current assays (Dube, & Stein, 2018; Ryscavage *et al.,* 2014), to improve overall function of immunity as demonstrated by an increase in CD4+ T cell count (Ryscavage *et al.,* 2014), to prolong survival (Dube & Stein, 2018; Kemnic &Gulick, 2019; Ryscavage *et al.,* 2014), to reduce deaths and illnesses associated with HIV (Kemnic &Gulick, 2019; Ryscavage *et al.,* 2014), to better the quality of life of people living with HIV [PLWH], and to lower the risk of transmission of HIV from PLWH to others (Kemnic & Gulick, 2019). This makes ART the most reliable option and the cornerstone of HIV treatment and prevention to-date (AIDSinfo, 2017; Günthard *et al.,* 2016; Lenjiso *et al.,* 2019; Sonali *et al.,* 2019). Research has demonstrated that early diagnosis of HIV and timely ART initiation reduced infant mortality and progression to disease by approximately 76% and 75% respectively (Gaitho *et al.,* 2021). Gill *et al*. (2017), demonstrated that infants tested and placed on prophylaxis immediately after birth had lower probabilities of HIV infection via vertical transmission compared to those tested and treated a month or two after birth. The case of the "Missisipi baby" whose HIV viral load went undetectable for 27 months as a result of early antiretroviral therapy was a great milestone towards the ratification of the use of ART for treatment and prevention of HIV (AIDSinfo, 2017). Further evidence on reduced mortality and reduced risks of HIV infection have been reported in several other studies with millions of HIV related deaths being averted over the years (Ferrand, 2017).

Recent efforts have aimed to scale-up the provision of ART within the constraints of resource-limited settings (National AIDS Control Council, 2018; Teasdale *et al.,* 2020) with global access to ART significantly increasing to estimated 17 million people in 2016, up from 1.3 million people in 2006 (Teasdale *et al.,* 2020). As antiretroviral services are decentralized, providing access to treatment for many more patients, there is a need to assess the effectiveness of standard regimens delivered under routine programmatic conditions that exclude the use of expensive and unavailable laboratory

testing (Teasdale *et al.,* 2020). Access to antiretroviral therapy ART is an integral component of the integrated HIV patient care and management (Bulstra *et al.,* 2021; World Health Organization, 2016). The HIV therapy boosts the patients' immune system, reduces the rate of illness from opportunistic ailments, raising the patients' quality of life by reducing morbidity and mortality (Cohen *et al.,* 2016). Evidence shows that ART prevented approximately 6.6 million AIDS related deaths worldwide, including 5.5 million deaths in low and middle-income countries between the years 1996 and 2012 (Bulstra *et al.,* 2021; Zhou *et al.,* 2016). In addition, ART has been shown to reduce the risk of HIV transmission (Teasdale *et al.,* 2020; Zhou *et al.,* 2016).

The rapid scale-up of antiretroviral treatment since 2010 by many of the world's high burden countries has reduced AIDS-related deaths globally from 1.5 million cases in 2010 to 1.1 million cases in 2015 (United Nations AIDS, 2016), with a further decrease in the annual number of AIDS related deaths to 680,000 in 2020 (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2021). As more nations adopt the updated guidelines from the WHO to treat every person diagnosed with HIV immediately after diagnosis (commonly referred to as test and treat strategy in many clinical circles in Kenya), there is evidence of individual and community public health benefits (Bain *et al.,* 2017; Joint United Nations Programme on HIV/AIDS (UNAIDS), 2016). ART coverage scale-up has been on a fast-track trajectory that has surpassed expectations (Bain *et al.,* 2017; Teasdale *et al.,* 2020). In low- and middle-income countries (LMICs), where over 90% of the HIV-infected population is found, there has been a drastic expansion of ART coverage in the last decade, with global treatment coverage reaching ~73% of all people living with HIV by the end of 2020 (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2021).

The continuous increase in ART coverage enhances the achievement of the 95–95–95 WHO treatment targets by 2030, whose objective is to diagnose 95% of all HIV sero-positive persons, provide antiretroviral therapy (ART) for 95% of those diagnosed, and achieve viral suppression for 95% of those treated by the year 2030 (Bain *et al.,* 2017; Joint United Nations Programme on HIV/AIDS (UNAIDS), 2016). Kenya being one of

the low income countries highly burdened by the HIV epidemic (Kharsany & Karim, 2016; Okal *et al.,* 2020), has witnessed an increasing ART among HIV infected people over the years in accordance with the 95–95–95 treatment target (Bain *et al.,* 2017; Kharsany & Karim, 2016; Joint United Nations Programme on HIV/AIDS (UNAIDS), 2016). It has been estimated that 423,000 AIDS-related deaths were averted between 2004 to 2015 as a result of the scale-up of ART in Kenya (Bain *et al.,* 2017; Kharsany & Karim, 2016).

The discovery and development of antiretroviral therapy against HIV has made significant progress in recent years with the emergence of new and different classes of drugs against HIV (Günthard *et al.,* 2016; Seitz, 2016). The combination of three or more of these anti-HIV drugs into multidrug regimens, often termed as highly active antiretroviral therapy (HAART) have been shown to inhibit replication of HIV to achieve low or undetectable levels of HIV in circulation (Rodger *et al.,* 2016). One of the most significant advances in the management of HIV since its discovery is the treatment of HIV infected patients with anti-HIV drugs, with potency to suppress HIV to undetectable levels (Seitz, 2016). Since the first HIV specific anti-HIV drugs were given as monotherapy consisting of only one drug, the standard of HIV care has improved greatly to include cocktails or combinations of anti-HIV agents (Günthard *et al.,* 2016; Seitz, 2016). The advent of HAART combination therapy for the HIV treatment was groundbreaking in drastically reducing the morbidity and mortality associated with HIV infection and its associated disease; AIDS (Kemnic & Gulick, 2019; Seitz, 2016). Combination ART suppresses viral replication and reduces the plasma HIV viral load to below detectable limits for the most sensitive testing platforms (<40 RNA copies/mL) resulting in a significant reconstitution of the immune system (Kyeyune *et al.,* 2016). By mid-1990's, it was confirmed that combination therapy with two nucleoside analogues is better than monotherapy in reducing levels of HIV RNA levels in blood, increasing CD4 cell counts, and preventing progression to disease and death (Kemnic & Gulick, 2019). According Kemnic and Gulick, (2019), HAART has been shown to significantly reduce the mortality rate and increase the life span of HIV-infected patients by

maintaining viral loads below levels of detection, thus preventing the onset of AIDS and other related diseases.

Since the discovery of HIV, an arsenal of over 28 anti-HIV drugs already approved by the United States' Food and Drug Administration (US FDA)are available for treatment of HIV and other associated illnesses (Kemnic & Gulick, 2019; AIDSinfo, 2017; Kyeyune *et al.,* 2016; Deeks *et al.,* 2015). These drugs are grouped into eight distinct classes based on their molecular mechanism of action, the stage of HIV life cycle they target and their associated drug resistance profiles: nucleoside-analog reverse transcriptase inhibitors (NRTIs), non–nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, protease inhibitors (PIs), fusion inhibitors, CCR5 co-receptor antagonists, Post-attachment inhibitors, and Pharmacokinetic Enhancers (Kemnic & Gulick, 2019; Krauß & Bracher, 2018).

2.7 Mechanism of action antiretroviral drugs

The HIV reverse transcriptase enzyme plays a key role in its life cycle and has been a major target for drugs against HIV (Kemnic & Gulick, 2019; Seitz, 2016). RT inhibitors are classified two main classes acting by distinct mechanisms as follows: Nucleoside RT inhibitors (NRTIs) are characterized by the lack of a 3' hydroxyl group on the ribose moiety and thus act as chain terminators during the reverse transcription of HIV RNA (Kemnic & Gulick, 2019; Deeks *et al.,* 2015). NRTIs first enter the cell and are thereafter converted to the triphosphate form (nucleoside RT inhibitor triphosphates [NRTI-TPs]) by kinases inside the host cell (Kemnic & Gulick, 2019). Because the NRTI-TPs are analogs of the normal dNTPs, NRTI-TPs are incorporated into the primer strand by RT (Seitz, 2016; Deeks *et al.,* 2015) However, because the NRTI-TPs do not have a 3′-OH group on the sugar moiety, a NRTI monophosphate (NRTI-MP) incorporated into viral DNA inhibits continued DNA synthesis and the primer chain is thereby terminated (Holec *et al.,* 2017). The NRTIs, usually in their inactive form, resemble nucleosides and are activated by the host to convert them to their active triphosphate form (Waller & Sampson, 2018). After binding the active site, their lack of

lack of 3’ hydroxyl group leads to the termination of the polymerization process (Holec *et al.,* 2017). NRTIs have however been shown to interfere with the host mitochondrial DNA synthesis as well as other anti-retroviral drugs (Holec *et al.,* 2017; Waller & Sampson, 2018). The nucleoside analogues such as AZT, 3TC, ddI, ddC, d4T, and ABC contain a high affinity base (thymidine in the case of AZT) that attaches to a ribose sugar in which the normal 3’ hydroxyl gets replaced by an azido group (Holec *et al.,* 2017; Seitz, 2016).

NNRTIs bind to a hydrophobic pocket close to the polymerase enzyme active site thereby inhibiting the chemical step of the polymerization reaction during reverse transcription process (Holec *et al.,* 2017; Kemnic & Gulick, 2019). NNRTIs act by blocking HIV replication by inhibiting the RT enzyme from completing reverse transcription of the HIV viral single-stranded RNA genome into pro-viral DNA (Waller & Sampson, 2018). Usually, NNRTIs bind near the active site of RT enzyme and alter its ability to change its conformation (Holec *et al.,* 2017; Waller & Sampson, 2018). The above leads to increased enzyme rigidity prevents its normal polymerization function (Waller & Sampson, 2018). NNRTIs bind non-competitively to a hydrophobic binding pocket within the active site of the RT enzyme (Holec *et al.,* 2017).

Maturation of HIV after release from the host cell involves cleavage of viral *Gag* polyprotein precursors into smaller functional proteins by protease enzyme before proceeding to infect new cells (Freed, 2015; Zulfiqar *et al.,* 2017). Protease enzymes encoded by HIV offer a unique and attractive target for preventing HIV maturation (Freed, 2015). HIV protease enzyme is a symmetrical aspartyl dimer with a central core that binds the peptide that are candidates for modification by the enzyme (Freed, 2015; Zulfiqar *et al.,* 2017). PIs target and inhibit the enzymatic activity of the HIV protease enzyme activity thereby preventing the cleavage events in *Gag* precursor polypeptides resulting in the production of premature non-infectious HIV particles (Freed, 2015; Zulfiqar *et al.,* 2017). PIs remain a key component of HIV patient treatment regimens to-date especially due to their high genetic barrier (Zulfiqar *et al.,* 2017). Currently, there are over ten HIV PIs approved for HIV management by the FDA; saquinavir, indinavir,

ritonavir, nelfinavir, amprenavir, fosamprenavir, lopinavir, atazanavir, tipranavir, and darunavir with lopinavir and atazanavir finding greater use in most sub-Saharan Africa (Zulfiqar *et al.,* 2017). Long-term treatment side-effects associated with PIs include HIV protease inhibitor-induced metabolic syndromes such as dyslipidemia, insulin-resistance, and lipodystrophy/lipoatrophy, cardiovascular and cerebrovascular diseases and poor bioavailability (Marin *et al.,* 2021). Therefore, the development of better, safer and potentially promising protease inhibitors is an urgent need (Marin *et al.,* 2021).

HIV integrase enzyme catalyzes the 3′-processing of pro-viral DNA terminals, and the integration of the processed 3' ends into host DNA (Richetta *et al.,* 2019). Integrase inhibitors (IIs), are a class of anti-HIV drugs targeting the integrase enzyme and are potent against viruses resistant to other ARV classes, such as NRTIs and NNRTIs, PIs and fusion inhibitors (Richetta *et al.,* 2019). Raltegravir (RAL) and has therefore not found much use clinically (Anstett *et al.,* 2017). Dolutegravir (DTG) are the major IIs in use today (Kemnic & Gulick, 2019).

2.8 Development of HIV drug resistance against antiretroviral therapy.

There are several anti-HIV multidrug therapies targeting the different stages of the virus' life cycle but whose effectiveness has been compromised by drug-resistance mutations within the viral genome (Günthard *et al.,* 2016; Reid-Bayliss & Loeb, 2017). As a result of the error-prone HIV reverse transcription process occasioned by the lack of proof reading capability of the RT enzyme, it is estimated that a single mutation is introduced for every 1,000–10,000 nucleotide bases synthesized (Günthard *et al.,* 2016; Reid-Bayliss & Loeb, 2017). Since HIV has a high rate of mutation, there is a frequent emergence of drug resistance with long-term ART use (Reid-Bayliss & Loeb, 2017). Considering that the HIV genome is ∼10,000 base pairs in length, 1-10 mutations may be generated with every replication cycle for each viral genome (Seitz, 2016). As a result of this enormous potential for generating genetic diversity, HIV variants with reduced susceptibility to any one or more anti-HIV drugs pre-exists in the viral quasi-species even before initiating therapy (Günthard *et al.,* 2016; Seitz, 2016). One strategy

employed to improve the efficiency antiretroviral therapy has been the use a combination of agents that inhibit different steps in the HIV life cycle (World Health Organisation, 2019). Combinations of different classes of ant-HIV drugs into a single regimen per dose per individual are the basis for an effective ART that halts, suppresses and slows down viral replication, leading to partial immune system recovery and a reduction in AIDS related morbidity and mortality (Kyeyune *et al.,* 2016).

With the increasing global access to ART in recent years, evidence has shown that between 8% and 23% of patients will fail first-line ART within the first 5 years after initiation (Lenjiso *et al.,* 2019). Patients failing first-line medications need to be switched to more expensive PI based second-line ART regimens (Joram *et al.,* 2017), though majority of patients on ART are expected to achieve viral suppression within one year of treatment initiation (Joram *et al.,* 2017). Three categories of HIV drug resistance (HIVDR) have been described: Transmitted HIVDR (TDR) which occurs when an uninfected ARV-naive person is infected with an already drug-resistant virus (Lavu *et al.,* 2017; Silverman *et al.,* 2017; World Health Organization, 2017). Globally, most TDR is found in people who develop HIVDR and who transmit resistant virus to previously uninfected people who are yet to start medication (Lavu *et al.,* 2017; World Health Organization, 2017). TDR greatly affects the test and treat programs which are critical in the management, prevention and control of HIV (Silverman *et al.,* 2017). Acquired HIVDR (ADR) occurs when HIVDR associated mutations develop as a result of the presence of ART (Clutter *et al.,* 2016; Hamers *et al.,* 2018; Lavu *et al.,* 2017; World Health Organization, 2017). ADR may emerge as a result of suboptimal adherence to medication, treatment interruptions, inadequate plasma drug concentrations, or the use of suboptimal drugs / drug combinations (World Health Organization, 2017). Pre-treatment HIVDR (PDR) refers to resistance that develops in individuals newly starting ART and can be either TDR or ADR due to previous ART drug exposure (Clutter *et al.,* 2016; Hamers *et al.,* 2018). Examples of people who may possess PDR include women who have received ARVs for PMTCT or people who have

previously had ART and defaulters who reinitiate therapy after defaulting (Clutter *et al.,* 2016; Lavu *et al.,* 2017).

The phenomenon of HIVDR in many low-income countries especially in sub-Saharan has been compounded by poor laboratory infrastructure, inadequate technical skills among staff at most testing laboratories, insufficient funding and incompetent procurement and delivery systems (Clutter *et al.,* 2016; Silverman *et al.,* 2017). Resistance of ART is an important cause of therapeutic failure in patients receiving antiretroviral therapy and therefore worth monitoring (World Health Organization, 2017). In spite of advances in ART coverage that have transformed HIV care and management as well as the control of the spread of regional epidemics, HIVDR to ART has emerged in all locales in which such drugs are used (Silverman *et al.,* 2017).

One of the greatest concerns surrounding the management of HIV disease in Kenya and other low-income countries is the high rate of poor adherence brought about by a combination of various factors including: disruptions in supplies of ART medication, wrong dosage of drugs, patients "forgetting" to take drugs frequent change of medication, interruptions in treatment due to financial constraints, and stigma thus increasing the chances of medication default rates (World Health Organization, 2017). As ART scale-up for the treatment and prevention of HIV continues globally, there is a high likelihood of increase in the levels of HIVDR (World Health Organization, 2017).

There are limited treatment options in Kenya and other high burden sub-Saharan African countries and therefore it is likely that if HIVDR develops, future ART options in newly infected individuals will most likely be compromised (Silverman *et al.,* 2017; World Health Organization, 2017). Treatment of a transmitted drug-resistant HIV variant is associated with an increased risk of virologic failure, higher mortality and morbidity (Silverman *et al.,* 2017; World Health Organization, 2017).

2.9 Determination of HIV treatment failure in Kenya

HIV treatment failure in Kenya is determined using the following criteria: New or recurrent WHO stage 4 condition or certain WHO clinical stage 3 AIDS defining illnesses such as. tuberculosis and karposis sarcoma, which is also referred to as clinical failure, a rise in plasma HIV viral load above 1,000 copies/ml (virologic failure), and a concomitant fall in CD4 count to baseline or persistent CD4 levels below 250 cells/mm^3 also referred to as immunologic failure (National AIDS Control Council Kenya,, 2016). Biologically, virologic failure occurs first and earlier in infection, followed by immunologic failure, then clinical failure (National AIDS Control Council Kenya,, 2016).

2.10 HIV viral load testing among people living with HIV in Kenya

Viral load is the main and most sensitive prognostic tool currently employed in the identification of treatment failure in the Kenyan programmatic settings (Joram *et al.,* 2017). Lower rates of HIVDR have also been associated with routine viral load monitoring programs in which early detection of virological rebound provides the opportunity for adherence counseling or regimen modification as necessary, prior to the evolution of multiple DRMs (Mwau *et al.,* 2018). Identification of people with treatment failure and their switch to second-line regimens is one of the key performance indicators used to measure the success of antiretroviral treatment (ART) programs (Patel *et al.,* 2020). HIV viral load is a test a blood test that measures the amount of HIV in a sample of your blood (Mwau *et al.,* 2018). Viral load testing has found wide utility in Kenya and many other high HIV burdened countries around the world in the past few years for assessing viral response to antiretroviral therapy and identification of patients failing treatment (Cherutich *et al.,* 2016; Mwau *et al.,* 2018; Patel *et al.,* 2020). In 2016, the Kenyan government introduced guidelines outlining the requirements of viral load testing for ART monitoring and identification of treatment failure, recommending that viral load testing should be performed at 6 and 12 months after ART initiation annually thereafter if the result is less 1,000 copies/ml (Mwau *et al.,* 2018; Patel *et al.,* 2020;

Sandbulte *et al.,* 2020). These guidelines define HIV treatment failure as a persistently high viral load ≥ 1,000 copies/ml for two consecutive viral load tests performed within a 3-month interval with adherence support between the two tests) at least 6 months after ART initiation (Ministry of Health & National AIDS and STI Control Programme, 2016; National AIDS and STI Control Programme, 2018). Persistently high viral load above 1,000 copies/ml of blood despite being on ART and undergoing adherence counseling constitutes virologic failure (Mwau *et al.,* 2018; Patel *et al.,* 2020).

In Kenya, routine laboratory monitoring of HIV viral load is part of the basic standard of care package offered to people living with HIV (PLHIV) in Kenya, and consists of tests used to monitor the efficacy of ART in ensuring viral suppression [CD4 and viral load] (Joram *et al.,* 2017). Clinical failure is defined as the presence of a new or recurrent WHO stage 3 or 4 disease in HIV infected individuals after being on ART for at least 6 months (Joram *et al.,* 2017; National AIDS Control Council Kenya, 2016), while immunologic failure refers to a CD4 count decrease by more than 30% from peak or failure of CD4 count to rise to more than 100 cells/mm^3 12 months after initiation of ART (Joram *et al.,* 2017; National AIDS Control Council Kenya, 2016). Virologic failure occurs when a repeat viral load remains persistently above 1000 copies/ml after three months of adherence counselling or after an initial viral load test (Joram *et al.,* 2017). The Kenyan Ministry of Health (MOH) currently recommends the use of viral load monitoring to identify treatment failure for patients on ART (Joram *et al.,* 2017). The MOH has fully adopted routine viral load monitoring and disregarded the use of CD4 testing for confirmatory testing in all health facilities offering HIV management, care and treatment in Kenya (Joram *et al.,* 2017). Despite viral load being a sensitive tool for detecting treatment failure, it has been shown that some individuals could exhibit virologic failure without any genetic failure especially due to poor adherence to medications leading to misdiagnosis of HIVDR (Zhou *et al.,* 2016). HIVDR misdiagnosis may cause misclassification of treatment failure leading to early and unnecessary switching to second line regimen which is costly and limits choices for later switches in case of confirmed HIVDR (Häggblom *et al.,* 2017).

2.11 HIV drug resistance testing among people living with HIV in Kenya

There are two general types of HVIDR testing assays used in clinical practice: genotypic assays which involve HIV genetic sequencing to detect mutations that confer HIVDR and phenotypic assays; cell culture–based viral replication assays either in the absence or presence of anti-HIV drugs (Cheung *et al.,* 2016). Genotypic resistance assays are used to identify specific codon mutations in the reverse transcriptase and protease genes of plasma virus by amplification and sequencing or by use of standardized probes (Cheung *et al.,* 2016; Silverman *et al.,* 2017). Results obtained are then compared with databases that match particular mutations with drug resistance. Phenotypic HIVDR assays measure the drug susceptibility of the virus by determining the concentration of drug that inhibits viral replication in tissue culture (Weng *et al.,* 2016).

Kenya has made significant progress in the provision of genotyping for HIVDR with both the public and private institutions being involved in training personnel and acquiring the relevant platforms required to carry out HIVDR testing over the years (National AIDS Control Council, 2018). It is recommended that HIV drug resistance genotyping data should be transferred electronically from the resistance genotyping laboratory to the national data centre in the form of a nucleotide sequence text file or files. The database is important to keep record on the sequences obtained (National AIDS Control Council, 2018). However, due to the cost limitations, resistance testing is currently limited mainly to research institutions, the private sector and a selected number of Universities in Kenya (Cherutich *et al.,* 2016; National AIDS Control Council, 2018).

2.12 Combination of HIV viral load and drug resistance testing to determine treatment failure among people living with HIV in Kenya

A combination of HIV viral load testing and drug resistance testing has been shown to ensure long-term success of global ART programs hence improvement of treatment outcomes (Silverman *et al.,* 2017). Depending on viral load testing alone to measure treatment failure may lead to misdiagnosis of treatment failure which could lead

unnecessary regimen switch (Häggblom *et al.,* 2017). Since drug resistance mutations appear long before virologic failure, resistance is detected more often in patients with infrequent viral load testing because VF is usually detected later, after DRMs have had time to accumulate (Zhou *et al.,* 2016). Drug resistance is a more specific measure of treatment failure which is able to effectively discriminate adherence related treatment failure from drug resistance related failure (Zhou *et al.,* 2016). Combination of HIV viral load and drug resistance testing reduces chances of misclassification of treatment failure leading to early and unnecessary switching to second line regimen which is costly and limits choices for later switches in case of confirmed HIVDR associated with viral load testing alone (Häggblom *et al.,* 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Busia County Referral Hospital, formerly known as the Busia District Hospital is a ministry of health facility located in Busia Municipality along Hospital road, Busia to Uganda Highway (Figure 3.1). This hospital in Busia offers 24-hour emergency medical care services including both inpatient and outpatient services and serves as the main referral hospital within Busia County. The Ministry of Health began treating patients in Busia County Hospital in July 2003. Busia County Kenya, with a population of approximately 823,504 has a HIV prevalence of 7.7% for age 15-49 years (National AIDS Control Council, 2018; World Health Organizatio, 2017). After a period of ART expansion and scale-up, the project is now focusing on strengthening care and treatment of patients. Most patients have been treated with the generic, standard fixed dose combination of TDF, AZT, 3TC, EFV and NVP (National AIDS and STI Control Programme, 2018). However, recent guidelines have seen a switch from both EFV and NVP to DTG except in children and women who are either expectant or planning to conceive (World Health Organisation, 2019). All treatment is provided free of charge to all patients at the hospital's Comprehensive Care Centre. Resistance testing is however not carried out routinely on these patients. Busia County Referral Hospital was the study site. Busia County Referral Hospital was preferred as the study site since it has a well-established HIV treatment programme. The hospital is also based at the busy transit and border town of Busia with cross-border town with high HIV prevalence in the area and its proximity to the border with Uganda, where the circulating HIV subtype profiles are different from the ones recorded in Kenya.

3.2 Study population

The study population was drawn from a group of patients currently receiving standard first-line ART through Busia County Referral Hospital. These patients attended routine

comprehensive care clinics at the Hospital. The patients included in the study were male and female HIV seropositive residents from the entire Western region of Kenya.

3.3 Study design

This was a cross-sectional study with purposive sampling of patients receiving standard first-line ART at the Busia County Referral Hospital for more than 12 months.

3.4 Inclusion criteria

3.4.1 Only on 1st line anti-HIV regimen (2 NRTI+1 NNRTI).

3.4.2 Patients aged 18 Years and above.

3.4.3 Patients who consented to participate.

3.4.4 Patients on ART for 12 months or more.

3.4.5 Patients who were initiated into 1st line ART at Busia County Referral Hospital.

3.4.6 Kenyan citizen.

3.5 Exclusion criteria

3.5.1 Children and adolescent patients (aged below 18 years).

3.5.2 ART naïve patients or those receiving other non-standard or second-line regimens (Table 3.1).

3.5.3 Patients who have been lost to follow-up (those who have not not attended the clinic within the previous 6 months).

3.5.4 Patients who did not give consent to participate in the study.

3.5.5 Patients transferred out to another program or organizations.

3.5.6 People who transferred from other treatment programmes.

3.5.7 Patients hospitalized at the time of the study with severe illness (HIV-related or otherwise).

3.5.8 Non-Kenyan citizen.

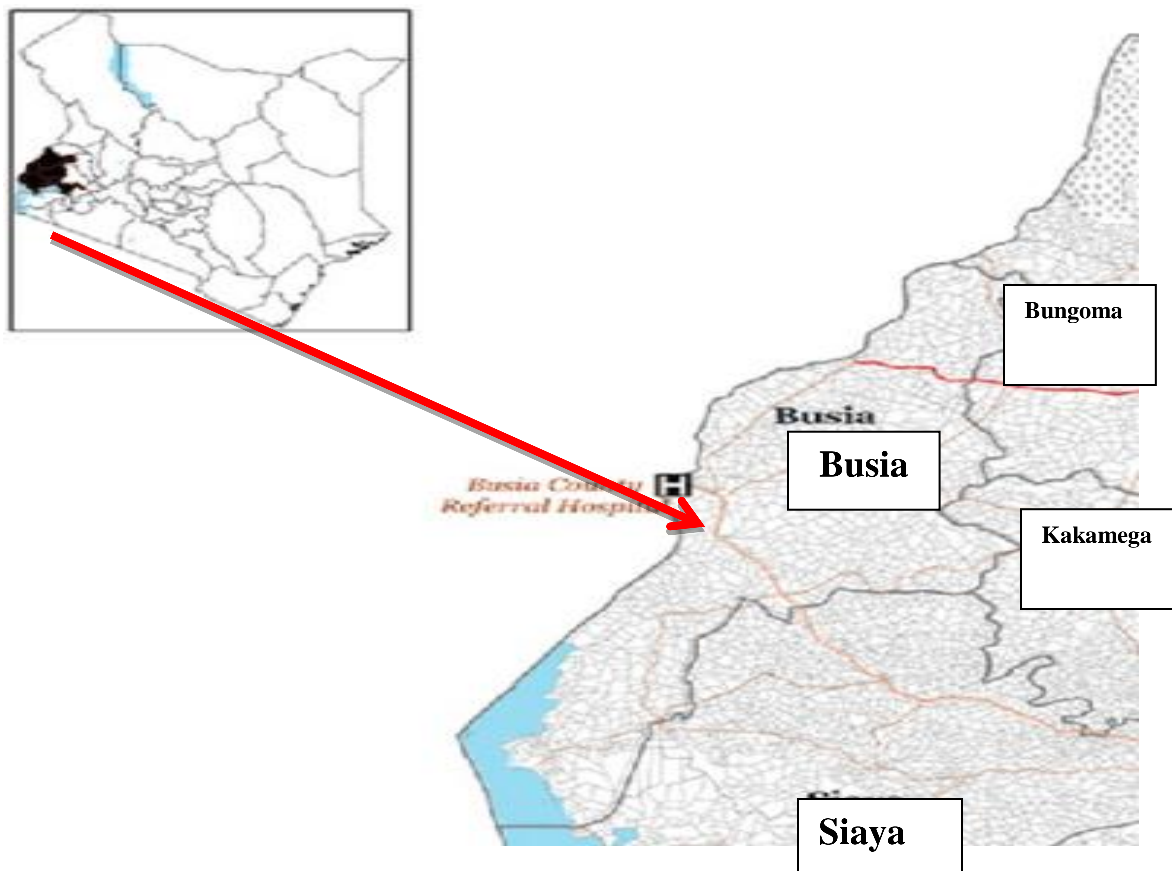


Figure 3.1: Map of Busia Count, Kenya showing Busia County Referral Hospital, the sampling site (Kenya National Bureau of Statistics, 2013).

3.6 Sampling criteria and sample size determination

Table 3.1: A summary of the number of participants eligible to the study

Category	Number of patients
Number of active patients 18 years or over receiving more than 12 months of first line ART	1103
Number of non-treatment naive patients at ART initiation	178
Number of eligible patients for viral load testing receiving more than 12 months of first line ART	925

Using a HIV prevalence of 5.0% in Kenya at the time, the sample size was derived using (Chadha, 2006) formula as shown below.

$$N = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$$

N = Minimum sample size giving power to the study, P = National prevalence of HIV (5.0%), d=absolute precision required on either side of the proportion. P and d are expressed in fractions, Z is a constant, and its value is 1.96 for 95% confidence. According to Kimanga *et al.,* (2014), prevalence of HIV in Kenya 2016 is 5.0%. Thus P=5.0% (0.05), Z=1.96, d=0.05

Therefore,

$$N = \frac{1.96^2 X\ 0.05(1-0.05)}{0.05^2}$$

= 75 Samples

Therefore, the minimum sample size needed for statistical representativeness of population and to provide power to the study through drug resistance testing was 75 samples. This is the minimum number of samples needed for drug resistance testing to provide power to the study.

The total number of patients who were 18 years and above receiving ART for 12 months and above at the Busia County Referral Hospital at the time of the study was 1103. However, 178 participants transferred from other treatment programmes and were excluded from the study as outlined in the exclusion criteria. Therefore, the number of patients enrolled to the study for viral load testing was 925.

Considering that the minimum sample size was 75 and that there was no maximum limit on the number of samples, all the 925 eligible participants were included in the study and had viral load testing carried out to determine those participants with detectable viral load (viral loads above the lowest limit of detection of 40 copies/ml and those with virologic failure (viral loads >1000 copies/ml). Only participants with virologic failure were allowed to proceed to drug resistance testing as per the standard guidelines. There were 146 participants with virologic failure but only 140 were successfully sequenced. Samples were collected from July 2016 over a period of 6 months.

3.7 Ethical considerations

This study was conducted in accordance with the Helsinki Declaration of 1975 on human subject research, (revised in 2000) and after approval by the Scientific Steering Committee (SSC) and the Ethical Review Committee (ERC) of the Kenya Medical Research Institute with ERC/SSC protocol number 1390 (Appendix III). Informed consent was sought from the participants before their samples were collected using a standard consent form (Appendix IV). Results of this study were made public via publications and presentations after the data analysis.

3.8 Benefits and risks to participants

Patients participating in the study benefited by the opportunity to have treatment failure identified earlier than if they were not in the study. Patients found to be failing ART, e.g. those with detectable viral loads and had drug resistance mutations, had their ART regimen changed to a more appropriate regimen. In general, the results of this study were used to improve program performance in Busia, benefiting all HIV sero-positive patients enrolled in the program. The risk from this study is the potential loss of privacy or confidentiality. However, since the results were treated in the same manner as all individual medical record information, this risk was not perceived to be any greater than that associated with being registered and receiving ART within the programme. Individual patient results were made available to both patients and treating clinicians through the normal process of access to medical records. The results from this study were made available to both health care workers and patients at the Busia County Referral Hospital through printed material and informal feedback sessions and published in peer reviewed journals.

3.9 Confidentiality of data and participant information

Data was collected and stored in Busia Referral Hospital as per normal clinical data collection criteria. Medical records were stored in a secure room in the HIV clinic and the database maintained on a computer without internet connectivity and internet in the same room. The database was also password protected. Delinked data (names removed) was transferred to the Centre for Virus research laboratories at the Kenya Medical Research Institute, Nairobi, where only study investigators had access to the data for analysis.

3.10 Laboratory testing procedures

3.10.1 Sample Preparation

Plasma samples for HIV viral load and drug resistance testing were stored at -70^{0}C. Patient demographic characteristics such as age, gender, and level of education were extracted from participant clinic files and data entered in MS-Excel spreadsheet database. HIV viral RNA testing was carried out to select samples with viral loads ≥ 1000 copies/milliliter (ml) for HIV drug resistance testing and genotyping. Since this was part of a larger study that required participants to provide 15 ml of blood, participants recruited to the study were required to give an additional 10ml of venous blood sample in addition to the 10 ml they initially provided for HIV viral load testing and HIV drug resistance testing. HIV viral load testing was aimed at providing an indication of treatment failure as per the standard of care while the HIV genotyping testing was aimed at detecting HIV genomic mutations that confer resistance to specific types of antiretroviral drugs as a tool for monitoring HIV treatment failure. HIV viral RNA was extracted from plasma, reverse transcribed into complementary DNA (cDNA) and subsequently amplified by a single tube polymerase chain reaction. This kit genotypes the entire HIV protease region from codons 1-99 of the *pol* gene and two-thirds of the *reverse transcriptase (RT)* region from codons 1-427 of the virus genome. Viral sequences were compared with a wild-type sequence in order to detect mutations using integrated systems including sample preparation, reverse transcription-polymerase chain reaction (*RT*-PCR), cycle sequencing module, negative and positive controls. The software was then used to convert continuous sequenced data for entire *PR* and 1-335 codons in *RT* region to a patient report. The genotypes were read and interpreted to outline the mutations present in the two codon sequences.

3.10.2 HIV RNA quantification (viral load) testing

Plasma samples stored at -70^{0}C were removed from the freezer and allowed to thaw at room temperature for 30 minutes. The thawed samples were briefly mixed by vortexing

and centrifugation at 300g for one minute. Sterile sarstedt screw cup tubes were labelled with the unique sample identifiers. Using a pipette and a sterile filtered pipette tip, 700 µl of plasma were transferred into the labelled sterile sarstedt screw cup tubes making sure that the sample from the storage cryovial was added into the corresponding sarstedt screw cup tube labeled with a similar unique sample identifier (ID) to avoid mix up. One tube was opened at a time to minimize chances of cross contamination. All these procedures were carried out in a biosafety cabinet with all universal safety measures being adhered to. The cryo-vials were placed into the automated Abbott M2000SP analyzer for HIV RNA extraction using magnetic bead technology. The HIV RNA extraction module was started from the attached computer. The specimen IDs were entered into the testing module as per the sequence on the racks. After two hours, extracted RNA collected on a PCR plate was transferred to the Abbott M2000 Real Time PCR machine for amplification and detection through fluorescence technology (Mwau *et al.,* 2018). Viral load results were downloaded from the computer attached to the PCR machine and entered into the excel spreadsheet for analysis. Each viral load run had three levels of controls (high positive, low positive and negative controls) included as per the requirements of good clinical laboratory guidelines for quantitative testing to ensure accuracy and reliability of results. The HIV RNA viral load testing equipment were calibrated and maintained following the manufacturer's instructions to ensure that they were in optimum working conditions.

3.10.3 Extraction of HIV RNA for drug resistance testing

Specimen with virologic failure (viral loads of >1000 copies/ml of blood) were selected and separated for HIV drug resistance testing. HIV viral RNA was extracted from these plasma specimens using the Qiagen® QIAamp RNA Mini Kit using the spin protocol according to the manufacturer's specifications (QIAGEN, 2012). The buffers were pre-prepared as described in the QIAamp RNA Mini Kit Handbook (QIAGEN, 2012). Five hundred and sixty (560) µl of prepared Buffer AVL containing carrier RNA were pipetted into a 1.5 ml micro-centrifuge tube. One hundred and forty µl plasma were

added to the Buffer AVL–carrier RNA in the micro-centrifuge tube and mixed by pulse-vortexing for 15 seconds. The mixture was incubated at room temperature (15–25^{0}C) for 10 minutes and briefly centrifuged to remove drops from the inside of the lid. Five hundred and sixty (560) µl of ethanol were added to the sample, and mixed by pulse-vortexing for 15 seconds before a brief centrifugation to remove drops from inside the lid. Six hundred and thirty (630) µl of the solution from step five were carefully added to the QIAamp Mini column (in a two ml collection tube) without wetting the rim, the cap closed and centrifuged at 7800g for one minute. The QIAamp Mini column was placed into a clean two ml collection tube, and the tube containing the filtrate was discarded. The QIAamp Mini column was carefully opened and 500 µl of Buffer AW1 added. The cap was carefully closed, centrifuged at 7800g for one minute and the QIAamp Mini column placed in a clean two ml collection tube and the tube containing the filtrate discarded. The QIAamp Mini column was carefully opened, 500 µl of Buffer AW2 added, the cap closed and centrifuged at full speed (24,000g) for three minutes. The QIAamp Mini column was placed in a clean 1.5 ml micro-centrifuge tube. The old collection tube containing the filtrate was discarded. The QIAamp Mini column was carefully opened and 60 µl of Buffer AVE equilibrated to room temperature added, the cap closed, and incubated at room temperature for one minute before being centrifuged at 7800g for one minute to elute viral RNA from the column to the bottom of the tube. Viral RNA obtained was stored at –20^{0}C till the time of analysis. Both positive and negative control were included for each round of extraction.

3.10.4 Reverse transcription of extracted HIV RNA for cDNA synthesis.

Reverse transcription of the RNA was performed by priming with UNINEF7 primer 5'-GCACTCAAGGCAAGCTTTATTGAGGCTT-3'(Khamadi *et al.,* 2005; Lihana *et al.,* 2009) close to the 3′ end of the viral RNA. All reagents and samples were thawed and reagents placed in a strata cooler previously stored at -20^{0}C to preserve their integrity. The extracted RNA (3 µl) was reverse transcribed in a total volume of 20 µl with 500 µM dNTP, 2.5 µM primer, 1X RT buffer, five mM $MgCl_2$, 10 mM DTT, 40 U RnaseOUT, and 400 U SuperScript^{TM} III RNase H^- RT (Invitrogen, Carlsbad, CA). The

RNA, primer and dNTPs were first incubated at 65^0C for five minutes, and the remaining reagents were added for cDNA synthesis at 50^0C for two hours, followed by a deactivation step at 85^0C for five minutes. Finally, two *U E. coli* RNase H (Invitrogen, Carlsbad, CA) was added, and the reaction tubes were incubated at 37°C for twenty minutes followed by 70^0C for fifteen minutes (Nadai *et al.,* 2008).

3.10.5 Nested polymerase chain reaction

The reverse transcriptase region of the viral genome was amplified using nested PCR from the cDNA to amplify the region of interest for HIV drug resistance analysis. All reagents and samples were thawed and reagents placed in strata cooler previously stored at -20^0C to preserve their integrity. The first-round of PCR had 25 µl reaction volume with a mixture containing three µl of five U Expand Long Template (Roche Diagnostics, Indianapolis, IN), 2.5 µl of 5X buffer, 0.3 µl of each RT18 and KS104 primers (Khamadi *et al.,* 2005; Lihana *et al.,* 2009) described in Table 3.2, 2.0 µl dNTP, 2.0 µl $Mgcl_2$, 14.7 µl of distilled water and 0.2 µl of *Taq* polymerase. The cycling conditions were 1 cycle of 95^0C for 10 minutes and 35 cycles of 95^0C for 30 seconds, annealing at 30^0C for 60 seconds, and 72^0C for one minute, and final extension of 72^0C for ten minutes. From the first-round PCR products, 3 µl was used as a template for the second reaction volume with the second set of primers in Table 3.2 (KS101 and KS102) (Khamadi *et al.,* 2005; Lihana *et al.,* 2009). The second cycling conditions were one cycle of 95^0C for 10 minutes and 35 cycles of 95^0C for 30 seconds, annealing at 68˚C for sixty seconds, and 72^0C for one minute, and final extension of 72^0C for ten minutes and the incubation at 68^0C in each cycle was for eight minutes. Annealing temperature for these primers was optimized at 60^0C (Khamadi *et al.,* 2005; Lihana *et al.,* 2009).

3.10.6 Analysis of PCR amplicons by gel electrophoresis

Upon completion of the second nested PCR, all products were viewed by conventional agarose gel electrophoresis (Khamadi et al., 2005) on a 0.8% agarose gel (SEakem LE® agarose; FMC BioProducts, Rockland, Marine, USA) in 1X TAE buffer (0.04M Tris

acetate, 0.001 M EDTA). Five µl ethidium bromide 0.5 µg per ml (Promega®, Madison, Wisconsin, USA) was added to the gel to stain the DNA (Sharp & Hahn, 2011). A 1 kb DNA molecular weight marker (Promega, Madison, Winsconsin, USA) was used to estimate the DNA band size.

Table 3.2: Primers used for polymerase chain reaction for the specific target gene region.

Primer	Primer Sequence	Reference
RT18: forward 1	5'GGAAACCAAAAATGATAGGGGGAATTGGAGG 3'	Khamadi *et al.,* 2005
KS104: reverse 1	5' TGACTTGCCCAATTTAGTTTTCCCACTAA 3'	Khamadi *et al.,* 2005
KS101:forward 2	5' GTAGGACCTACACCTGTTCAACATAATTGGAAG 3'	Khamadi *et al.,* 2005
KS102: reverse 2	5'CCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG 3'	Khamadi *et al.,* 2005

The samples were mixed with gel loading dye before loading into the respective wells on the gel. Electrophoresis was done at a constant voltage of one hundred volts/cm (100 V/cm) using a Mupid®2 plus submarine electrophoresis system power supply source. After electrophoresis, the location of PCR DNA fragments on the gels was visualized under an ultra violet light at a wavelength of 301 nm and photographed with the syngene^{TM} GeneGenuis computer system [Synoptics LTD, Cambridge, United Kingdom] (Khamadi *et al.,* 2005). With the UV light on, photos of the gel were taken using a camera mounted (Figure 3.2).

3.10.7 Sequencing of HIV positive specimen

The generated amplicons from the second nested PCR products, after analysis by gel electrophoresis were then directly sequenced using the second set of primers KS101 and KS102 (Khamadi *et al.,* 2005; Lihana *et al.,* 2009) described in Table 3.2**.** This sequence PCR was carried in a reaction mixture of 20 µl with a dilution ratio of 1:10. This contained 3 µl of DNA, 5X sequence buffer, 2.0 µl BigDye®, 10.5 µl of distilled water, and 1.5 µl of forward and reverse primers (Khamadi *et al.,* 2005; Lihana *et al.,* 2009).

Amplification was carried out using a thermal cycler at the following PCR conditions: denaturation for five minutes at 96^0C, and again for 10 seconds at 96^0C, annealing at 50^0C for five seconds, and a final extension at 60^0C for four minutes for twenty five cycles.

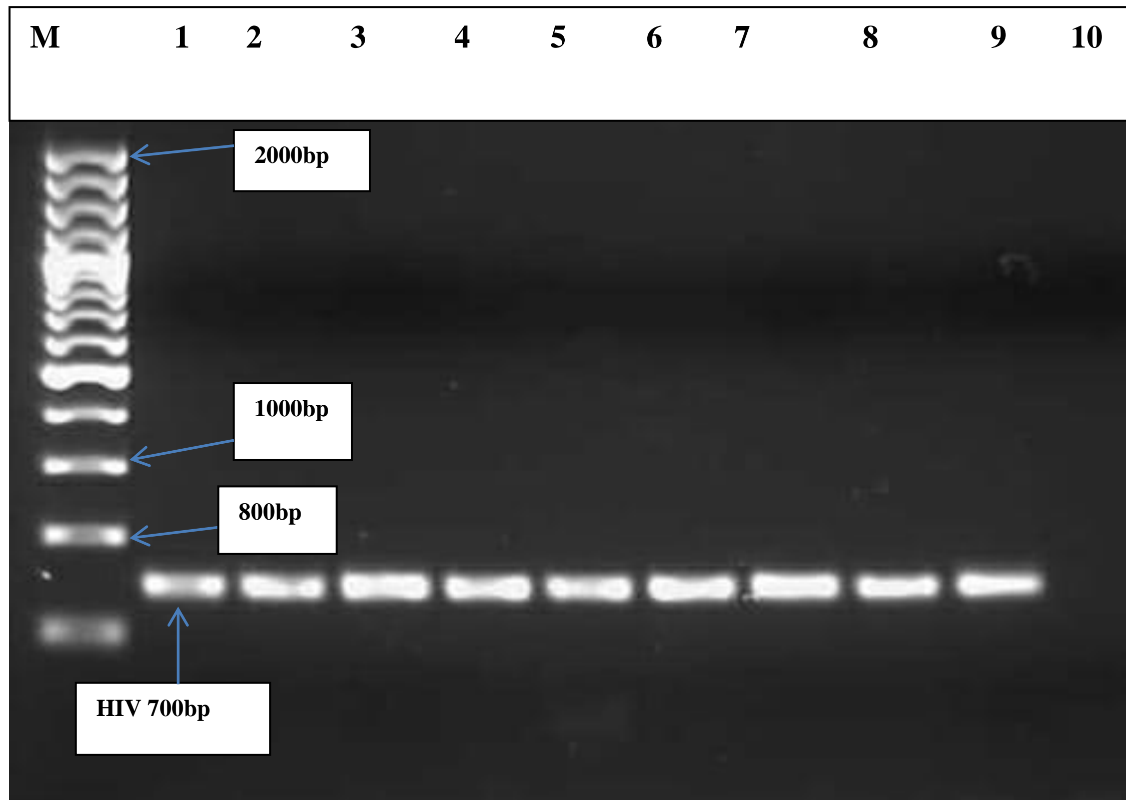


Figure 3.2: Gel electrophoresis image following reverse transcription showing HIV-1 at 700bp, sample bands and molecular markers (M represents the molecular weight marker, 1-8 represents patient samples, 9 represents the positive control and 10 represents the negative control).

The amplified products from the nested PCR were labeled in PCR reaction using the BigDye® sequence terminator kit from Applied Biosystems® and the products sequenced directly using an automated ABI 310 sequencer (Applied Biosystems, Foster City, CA). Using BigDye® sequence terminator kit, fluorescently labeled dyes are attached to ACGT extension products in DNA sequencing reactions. The dyes come in four colors red (labels Thymidine base), blue (labels Cytosine), black (labels Guanine)

and green (labels adenine). The dyes are incorporated using either 5'-dye label primers or 3'-dye label dideoxynucleotide terminators. AmpliTaq® polymerase was used for primer extension. The dyes were used to perform sequencing PCR with the template being the PCR products to be sequenced. This HIV-1 genotyping assay sequences the HIV-1 *pol* gene base pairs covering *PR* region (codons 1 - 99) and *RT* region (codons 1 - 247).

3.10.8 Sequencing reactions and purifications

For purification, two µl of three mM sodium acetate was added to an eppendorf tube, 50 µl of absolute ethanol and 20 µl of the dye-labelled PCR product were also added. This mixture was incubated at room temperature for fifteen minutes in the dark. The tube was centrifuged at 195 Xg for thirty minutes at room temperature. Five hundred (500) µl of 70% ethanol was added and centrifuged at 347g for five minutes and the supernatant discarded. The two steps above were repeated and DNA air-dried for a minimum of forty five minutes. Twenty (20) µl of template suppression reagent was added and heated at 95^0C for three minutes and then the contents transferred into sequencing tube and loaded on the automated ABI 310 DNA sequencer (Applied Biosystems, Foster City, CA) for direct sequencing. The sequences obtained were downloaded in FASTA format and stored for further analysis.

3.10.9 HIV sequence analysis

3.10.9.1 HIV sequence quality control

The sequences generated from section 3.10.8 were reviewed for completeness. A FASTA formatted file of the generated sequences was prepared for quality assurance. The FASTA formatted sequences were uploaded for quality control (QC) to https://www.hiv.lanl.gov/content/sequence/QC/index.html?. An email address where the QC results were to be submitted was provided. The BLAST percentage identities were reviewed and the identity or near-identity to reference strains noted to detect any sequence contamination. The output was reviewed for unexpected subtypes and

compared with the BLAST results. The QC output results were checked for hypermutations to rule out any cases of hypermutants. Stop codons and frameshifts were checked to determine sequence quality. The Neighbor Joining tree pictogram was reviewed to identify any sequences clustering together with unexpected subtypes.

3.10.9.2 Submission of sequences to GENBANK using Bankit

For submission to the GenBank, the data was prepared in three files: a sequence file in the FASTA format containing the 140 sequences, and a source modifiers file in TXT format containing source information for the 140 sequences and a feature table in TXT format containing sequence features. The BAnkIt GenBank entry generation tool was opened, the sequence information, manuscript information and annotation data, the three files previously generated were uploaded (FASTA and two .txt files) and the "Submit" button clicked to submit the sequences to GenBank for accessioning.

3.10.9.3 HIV drug resistance associated mutations

The nucleotide sequences were manually edited and individually submitted in Stanford HIV drug resistance database at (http://hivdb.stanford.edu/). The output file comprising of the circulating HIV subtype, HIV drug resistance associated mutations (DRAMs) profiles, phenotypic drug resistance and the mutation scores for PIs, NNRTIs and NRTIs were copied to a word document as the result to be disseminated to the clinicians for patient management. Genotypic drug resistance in the protease and *RT* regions were defined as the presence of one or more resistance-related mutations, as specified by the consensus mutation figures of the International AIDS Society-USA and the online tool on the Stanford HIValg-Software at https://hiv.grade.de. Data for circulating HIV subtype, HIV DRAMs profiles, phenotypic drug resistance and the mutation scores for PIs, NNRTIs and NRTIs was entered into the results database.

3.10.9.4 HIV subtyping using REGA HIV subtyping tool

HIV subtyping was performed using the automated REGA HIV Subtyping Tool Version 3.0 by opening the link https://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/. The FASTA formatted sequences were copied and pasted into the REGA HIV Subtyping Tool Version 3.0 and submitted for subtype analysis. The subtypes obtained and their proportions were downloaded in excel format.

The sequences were also pasted on to the Los Alamos BASIC BLAST tool on https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html. The subtypes obtained were transcribed on to an excel spreadsheet for further analysis. Similarly, the sequences were pasted on to the University of Stanford HIV drug resistance program version 8.8 on https://hivdb.stanford.edu/hivdb/by-sequences/. The HIV subtypes obtained were downloaded on to the excel spreadsheet pending analysis.

The HIV subtypes from the three subtyping tools were compared and analyzed for concordance and discordance.

3.10.9.5 Multiple sequence alignments using MEGA version 11.0

Multiple sequence alignments were carried out using MUSCLE in the Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0. The MEGA version 11.0 Alignment Explorer was launched by selecting Alignment -> Alignment/MUSCLE on the software window. A window appeared with the following options and the option “Create a new alignment” was selected. The unaligned sequences were copied and pasted from the text file to the Alignment Explorer. All the sequences were highlighted by selecting Edit -> Select All. The highlighted sequences were aligned by selecting Alignment -> Align by MUSCLE. The current alignment was saved as an alignment session file with the extension “.mas”, by selecting Data -> Export -> Save. The current alignment was saved as a MEGA file by selecting Data -> Export -> Save as MEGA file to allow it to be analyzed by MEGA.

3.10.9.6 Phylogenetic tree construction using MEGA version 11.0

The Phylogeny -> Construct Tree -> Maximum Likelihood commands were selected to display the analysis preferences dialog box on the MEGA software version 11.0 (Kumar *et al.,* 2016). The dialogue box popped up and the following important parameters were set: Statistical Method: Maximum Likelihood, Test of Phylogeny: Bootstrap Method, No. of Bootstrap Replications: 1000, Substitutions Type: Nucleotide and Model/Method: Jukes-Cantor model. All the other parameters were set at the default. Phylogenetic tree construction was initiated by clicking "OK". Once the computation was complete, the Tree Explorer appeared and display two tree tabs: the original Maximum Likelihood tree and the Bootstrap consensus tree. The results were exported and saved as a .mas file for further review.

3.11 Statistical data analysis

For statistical analysis, descriptive statistics (median, interquartile range [IQRs], mean, and percentages) were used to summarize the demographic and clinical characteristics of the study patients in this study. Multivariable analysis was performed to measure the association of all predictors and outcomes. For the multivariable analysis, a logistic regression model was used for outcomes with demographic characteristics. The number of NRTI, NNRTI and PI DRAMs per person and the differences of their distribution in either EFV or NVP based regimen were analysed by Pearson's chi-square test. Susceptibility/resistance analysis within EFV or NVP based regimen (paired samples: susceptibility of different drug regimens) was analysed by using Wilcoxon signed-rank test. Pearson's correlation coefficients were calculated. A p-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Participant demographic characteristics

A total of 925 participants were included in the study after meeting the study inclusion criteria. Of the 925 participants included in the study, 548 (59.24%) were females while 377 (40.76%) were males (Table 4.1). Six hundred and eighty four (735) particcipants representing 73.9% of the study participants were on EFV containing regimens while 241 (26.1%) of study participants being on NVP containing drug regimen. A total of 915 participants had successful viral load results with 10 participants failing viral load testing. The participants' ages ranged between 21-67 years (IQR = 14.25) with a mean age of 38.79 years, a median of 44 years, and a mode of 38 years. Of the 915 eligible participants with successful viral load test, 218 (23.8%) had detectable viral loads (>40 copies/ml) while 691 (75.5%) had viral load counts below detection levels (<40 copies/ml). Of the 915 who had successful viral load results, 544 (59.5%) were females while 371 (40.5%) were males (Table 4.1). Virologic failure was reported in 16% (146/915) of the participants although only140 were successfully sequenced and tested for drug resistance. HIV-1 DRAMs were reported in 62% (87/140) of the participants.

4.2 HIV RNA quantitative (viral load) testing results among patients receiving HAART in Busia County, Kenya.

Overall, the VL counts (reported in HIV viral copies/ml of blood usually denoted as copies/ml) ranged from Not Detected (ND) to 1,234,454 copies/ml. Six hundred and ninety-seven (697) participants (76.2%) of the total 915 participants had viral load counts below the limit of detection (40 copies/ml) and were reported as Not Detected (ND).

Table 4.1: Socio-demographic and clinical characteristics among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

Characteristics	Number of Participants (%)
Sex (N=925)	
Male	377 (40.76%)
Female	548 (59.24%)
Age (N=925)	
<30 years	177
31-40 years	380
41-50 years	226
>50 years	142
Range	21-67
Mode	38
IQR	14.25
Mean	38.79
Treatment Regimen (N=925)	
NVP based regimen	241 (26.1)
EFV based regimen	684 (73.9)
Treatment combination (N=925)	
AZT+3TC+NVP	241 (26.1)
TDF+3TC+EFV	578 (62.5)
AZT+3TC+EFV	106 (11.4)
Successful HIV Viral Load Testing (N=915)	
Detectable viral load	218 (23.8)
Viral load below detection limit	697 (76.2)
Male	371(40.5)
Female	544 (59.5)
Minimum	Not Detected
Maximum	1,234,454
Detectable HIV Viral Load Counts (N=218)	
Range	54-1,234,454
Mean	62,542
Virologic Failure (N=146)	
NVP containing regimens	34 (23.6)
EFV containing regimens	112 (76.7)
Female	101 (69.2)
Male	45 (30.8)
Range	1,472 – 1,234,454
Mean	93,300.30

Two hundred and eighteen (218) participants (23.8%) of the total 915 participants with successful VL had detectable viral load counts (>40 copies/ml). For these 218 participants with detectable VL, 152 (69.7%) were females with 66 (30.3%) being males, the range was 54 - 1,234,454 copies/ml. The mean viral load count for those with detectable viral load was 62,542 copies/ml, a median of 4,914 copies/ml, a mode of 234 copies/ml, a minimum of 54 copies/ml, and a maximum of 1,234,454 copies/ml (Table 4.1). There was a significant difference between male and female participants having detectable viral load counts with more females reporting detectable viral loads compared to males (p=0.0002).

Out of the 218 participants with detectable viral loads, 146 (66.9%) had viral load counts of greater than 1,000 copies/ml and were therefore classified as having virologic failure. Overall, there was 15.9% virologic failure. Of the 146 participants with treatment failure, 101 (69.2%) were females whereas 45 (30.8%) were males (Table 4.1). From the results, it was noted that females were significantly more prone to virologic failure compared to males (p=0.0001). The mean age for participants with virologic failure was 39.1 years, the median was 38 years, the mode was 35 years, while the minimum and maximum ages were 23 and 67 years respectively. For this group with virologic failure, the mean VL was 93,300 copies/ml, the median was 23,838 copies/ml, the minimum VL was 1,472 copies/ml and the maximum was 1,234,454 copies/ml (Table 4.1). The interquartile range was 72,690.25 at 95% CI. Age brackets 41-45 years recorded the highest number of patients with virologic failure with a total of 28 participants having virologic failure (Figure 4.1). Majority of participants with virologic failure were below 45 years of age. Therefore, younger participants (below 45 years of age) were significantly at a higher risk of having virologic failure than their older counterparts (p=0.0001). Of the 140 specimens successfully sequenced, 112 (80%) were from participants who were on EFV based regimen while 28 (20%) were from participants on NVP based regimen. Interestingly, there were 53 (37.9%) participants with virologic failure that did not possess any drug resistance-associated mutations (DRAMs) and did not exhibit phenotypic drug resistance against any of the drug classes under study. Of

these, 43(81%) participants had viral loads of less than 10,000 copies per ml. This implies a lower probability of genetic and phenotypic treatment failure at viral loads below 10,000 copies per ml (p=0.003).

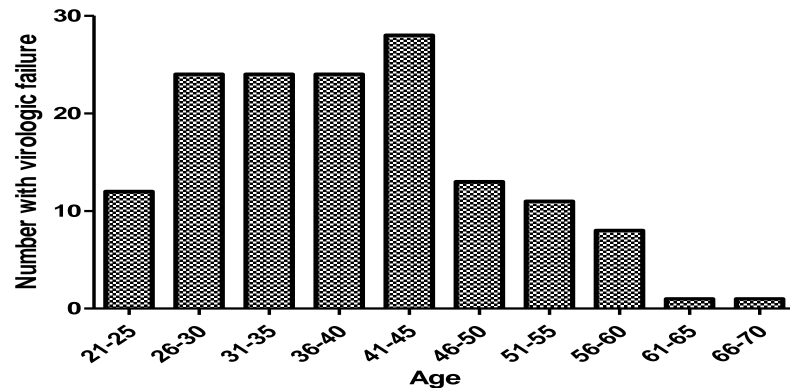


Figure 4.1: Number of study participants with virologic failure at different age brackets among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

4.3 Drug resistance associated mutations (DRAMs) among patients receiving HAART in Busia County, Kenya.

One hundred and forty-six (146) specimen were subjected to HIVDR testing to determine the presence of DRAMs. However, only one hundred and forty (140) sequences (95.8%) of the total 146 specimens that qualified for HIV drug resistance testing. They were successfully sequenced with six samples failing drug resistance testing. Out of the 140 specimen (GenBank accession numbers MW618176-MW618315- Appendix II) that were successfully sequenced, 87 (62%) sequences had at least one major HIVDR mutation against either the PIs, NNRTIs, NRTIs or a combination of two or more of the drugs in any of these classes and exhibited resistance against one or more antiretroviral drugs available in Kenya. Fifty three (53) participants (38%) were susceptible to all drug classes studied. Of these 87 participants with at least one drug resistance associated mutation (DRAMs), 65 (74.7%) were females while 22 (25.3%) were males. Consequently, there was a significant difference between females

and males in acquisition of any DRAMs with females being more vulnerable to having DRAMs (p=0.001). A total of 393 DRAMs against PIs, NNRTIs, and NRTIs were identified in the study population as follows: PI DRAMs =12 (3.1%), NNRTI DRAMs =197(50.1%) and NRTI DRAMs = 184 (46.8%) (Table 4.2). Out of the samples that were successfully sequenced, 53 (37.9%) did not possess any HIV drug resistance related mutations (did not have genotypic resistance) and were susceptible to all classes of anti-HIV drugs (did not exhibit any level of phenotypic resistance) despite exhibiting virologic failure.

There was no significant difference between presence of NNRTI and NRTI DRAMs in this population (p=0357). However, there were significant differences between the presence of NNRTI and PI DRAMs (p=0.0001) as well as between the presence of NRTI and PI DRAMs (p=0.0001). Comparatively, female participants had significantly higher rates of occurrence of specific DRAMs (p=0.0025); with 78.6%, 73.6% and 57.1% of women having DRAMs against NNRTIs, NRTIs and PIs respectively compared to males who had 21.4%, 26.4% and 42.9% mutations against NNRTIs, NRTIs and PIs respectively (Table 4.2). Consequently, female participants were significantly at a higher risk of acquiring either NNRTI, NRTI and PI DRAMs than males (p = 0.0462).

Participants were stratified into different age brackets. The age bracket 41-45 years recorded the highest number of DRAMs (38 participants), followed by age bracket 31-35 years, 36-40 years and 26-40 years (28, 27 and 24 participants respectively) as shown in Figure 4.2. When compared between different age brackets, there were highly significant differences in the probability of acquiring DRAMs with the ages 26-45 years having higher odds of acquisition (p=0.0001). Within a particular age bracket, there were significant differences in the odds of acquisition of the different classes of DRAMs with the population having higher probabilities of developing NNRTI and NRTI than PI DRAMs across the age brackets (p=0.0001).

Table 4.2: Number of NRTI, NNRTI and PI DRAMs among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

	Number of DRAMs	Number of patients with DRAMS		Number of patients without DRAMS		TOTAL
		Male	Female	Male	Female	
NRTI	184	30	47	32	31	140
NNRTI	197	29	53	33	25	140
PI	12	3	4	59	74	140
TOTAL	393	62	104	124	130	

Six sequences contained APOBEC mutations namely G99R, G51R, G68R, G190R, G86E and W229*. All the sequences were from patients on EFV based regimens with most being TDF+3TC+EFV, majority (4/6) being males, three subtype A1 and three subtype D (Table 4.3).

Table 4.3: Characteristics of patients with APOBEC mutations among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

Sequence ID	Gender	Age	Viral Load (copies/ml)	Regimen	REGA Subtype	APOBEC Mutation
SEQ043	Female	34	456274	TDF+3TC+EFV	A1	G99R
SEQ063	Female	39	24098	TDF+3TC+EFV	A1	G51R
SEQ068	Male	54	9807	AZT+3TC+EFV	A1	G68R, G190R
SEQ089	Male	50	4576	TDF+3TC+EFV	D	G86E
SEQ096	Male	45	65873	TDF+3TC+EFV	D	W229*
SEQ137	Male	43	203093	TDF+3TC+EFV	D	W229*

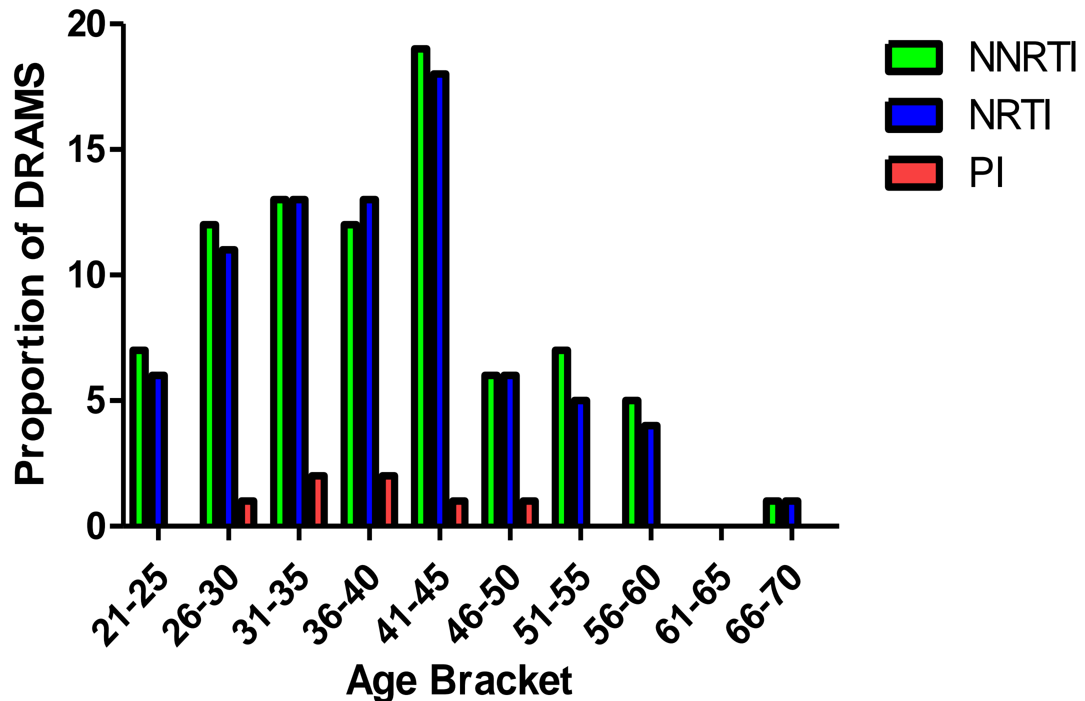


Figure 4.2: Number of NNRTI, NRTI and PI DRAMs among patients receiving highly active antiretroviral therapy in Busia County, Kenya grouped by age bracket.

4.3.1 NRTI DRAMs among patients receiving HAART in Busia County, Kenya.

A total of 184 different NRTI drug resistance associated mutations (DRAMs) that conferred resistance to ABC, AZT, D4T, DDI, FTC, 3TC, and TDF were identified in the study population. Out of the 140 specimens successfully sequenced, 77 (55%) sequences had at least one HIV-1 NRTI DRAM. M184I/V mutation was the most prevalent NRTI DRAM, present in 70 (90.9%) of all the patients with NRTI DRAMs. T215Y/F/S/I/N family NRTI DRAMs were present in 22 (12%) of the population. M41L, K70R/N and D67N/Y family RAMs were present in 17(9.2%), 16 (8.2%) and 14 (7.6%) respectively. On the other hand, K65R/Q and K219R/Q/E family DRAMs were present in 13 (7.1%) and 11 (6%) of the population respectively, whereas V75M/T, L74I/V and L210W were present in 7 (3.8%), 6 (3.3%) and 4 (2.2%) of the study

population respectively (Table 4.4). T69D DRAMs were present in 3 (1.6%) of the population. F77L was present in 1 (0.5%) of the population that possessed DRAMs against NRTIs (Table 4.4).

M184V/I DRAM present as the only NRTI DRAM conferred high level resistance to FTC and 3TC but rendered AZT and TDF susceptible. K65R mutation, though seldom present alone, did not have any effect on AZT but conferred high-level resistance to FTC and 3TC as well as intermediate level resistance to TDF. It is important to note that K65R only provided high level resistance to FTC and 3TC. In over 90% of the cases, K65R was found in combination with M184V/I mutations. There was only one case where K65R mutation was present alone and interestingly, this patient had high level resistance to TDF, intermediate resistance to FTC and 3TC (this is unlike the observation when K65R was found in combination with other NRTI DRAMs) but rendered AZT susceptible. In the presence of other NRTI DRAMs, K65R mutation provided intermediate resistance to TDF. Of the 13 patients who had K65R mutations, a majority of them (84.6%) had susceptibility to AZT. Ten (76.9%) of the 13 sequences with K65R were subtype A1 while 3 (23.1%) were subtype D.

Table 4.4: A summary of Nucleoside Reverse Transcriptase Inhibitor (NRTI) HIV drug resistance associated mutations in patients receiving HAART in Busia, Kenya

ART Regimen		N/140	Nucleoside Analogue Mutations (NAM)			Thymidine Analogue Mutations (TAMs) Type 1			Thymidine Analogue Mutations (TAMs) Type 2			
NNRTI	NRTI		M184	K65	L74	T215	M41	L210	D67	K70	K219	T69
Efavirenz (EFV) containing regimen												
EFV	TDF+3TC	101	52	10	5	15	9	1	12	13	7	0
	AZT+3TC	11	7	1	0	1	1	1	1	0	0	0
Nevirapine(NVP) containing regimen												
NVP	AZT+3TC	28	12	2	2	5	3	2	3	3	3	3
TOTAL		**140**	**71**	**13**	**7**	**21**	**13**	**4**	**16**	**16**	**10**	**3**

Thirty-seven (37) sequences had at least one type 1 or type 2 thymidine analogue mutations (TAMs). The main type 1 TAMs present were M41L, T215Y/F/I/S and L210W with M41L, T215Y/F/I/S being more prevalent than L210W. Type 2 TAMs present included D67N, K70R and K219Q/E with D67N/Y being the most prevalent. Presence of D67N/Y DRAM alone did not confer any resistance against the common NRTIs. Two patients had six different NRTI DRAMs, the highest number of mutations against this class of drugs recorded in this study. There were five sequences which exhibited DRAMs to NRTIs but did not possess any NNRTI DRAMs. Two of the five sequences also possessed PI DRAMs. Consequently, phenotypic drug resistance profiles against NRTIs were either susceptible, or contained either potential low-level resistance, low-level resistance, intermediate resistance or high level resistance in different proportions depending on the presence or absence of NRTI DRAMs, the type and combination of these DRAMs within the sequence (Figure 4.3).

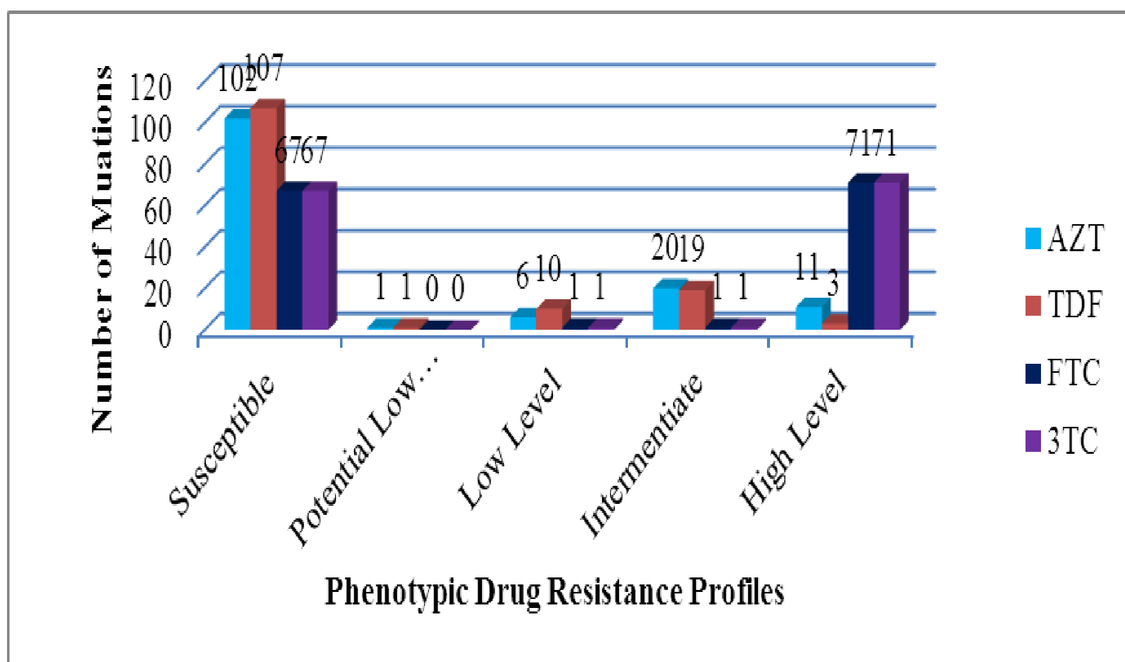


Figure 4.3: Number of resistance profiles for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: “Susceptible” - no evidence of reduced ARV susceptibility compared with a wild-type

virus. "Potential low-level resistance" – presence of mutations indicating previous ARV exposure or may contain mutation that are associated with drug resistance only when they occur with additional mutations. "Low-level resistance" - may have reduced ARV susceptibility. "Intermediate resistance" - high likelihood that a drug's activity will be reduced but that the drug will likely retain significant remaining antiviral activity. "High-level resistance" - patients infected with viruses having such mutations usually have little or no virological response to treatment with the ARV).

4.3.2 NNRTI DRAMs among patients receiving HAART in Busia County, Kenya.

A total 197 NNRTI DRAMs that conferred resistance to both first generation NNRTIs (EFV and NVP), and second generation NNRTIs (Etravirine and Rilpivirine) were present in the study population. Out of the 140 specimens successfully sequenced, 82 (58.6%) sequences had at least one HIV-1 NNRTI DRAM. K103N/S family of NNRTI DRAMs had the highest frequency being present in 39(19.8%) followed G190A/S family DRAMs which were present in 33(16.8%) of the population with NNRTI DRAMs. K101E/P, H221Y, Y181C/S/H, V108I and K238T family DRAMs were present in 20(10.2%), 18(9.1%), 17(8.6%), 16(8.1%) and 16(8.1%) respectively. A98G, E138A/K/G, P225H and V179D/T/E family DRAMs were present in nine (4.6%), six (3.0%), six (3.0%) and five (2.5%) respectively (Table 4.5). L100P/I, Y188N/F and M230L DRAMs were all present in four (2%) of the patients who exhibited NNRTI DRAMs. Comparison between EFV and NVP groups showed that there were significantly higher frequencies of NNRTI DRAMs in the EFV group compared to the NVP group (p=0.0032).

Presence of K103N/S in isolation conferred high level resistance to EFV and NVP, K103E alone did not confer resistance to any of the NNRTIs, while G190A mutation conferred intermediate resistance to EFV and high-level resistance to NVP. K101E/P was found almost always in association with K103N and/or G190A, the combination conferring high level resistance to both EFV and NVP. A98G and L100I mutations were not found in isolation. Y181C (which was mainly found associated with H221Y

provided intermediate resistance to EFV but high level resistance to NVP and intermediate resistance to both ETR and RPV.

Table 4.5: Summary of Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI) HIV drug resistance associated mutations in patients receiving highly active antiretroviral therapy in Busia, Kenya.

ART Regimen		N/140	Primary NNRTI Mutations							Secondary NNRTI Mutations							
NNRTI	**NRTI**		**K103**	**G190**	**Y181**	**K101**	**H221**	**V108**	**K238**	**A98**	**E138**	**P225**	**V179**	**L100**	**Y188**	**M230**
Efavirenz (EFV) containing regimen																
EFV	**TDF+3TC**	101	31	21	11	13	13	11	11	7	5	5	2	2	4	3
	AZT+3TC	11	1	7	2	4	1	1	3	0	0	1	0	0	0	0
SUBTOTAL																
Nevirapine(NVP) containing regimen																
NVP	**AZT+3TC**	28	7	5	4	3	4	4	2	2	1	0	3	2	0	1
SUBTOTAL																
TOTAL		**140**	**39**	**33**	**17**	**20**	**18**	**16**	**16**	**9**	**6**	**6**	**5**	**4**	**4**	**4**

Y188L mutation alone provided high level resistance to EFV, NVP and RPV but potential low level resistance to ETR. M230L provided high level resistance to ETR and RPV while M230I conferred low-level resistance to ETR and intermediate resistance to RPV. On the other hand, four participants had HIV DRAMs against NNRTI but did not possess any DRAMs against NRTIs. K103E DRAMs present in isolation did not confer any resistance against any NNRTI whereas V179D present alone conferred potential low-level resistance to ETR and RPV while providing high level resistance to EFV and NVP.

While fifty-eight participants did not possess any NNRTI DRAM, 21 participants had one NNRTI DRAM, 27 participants had two NNRTI DRAMs, 22 had three NNRTIs DRAMs, eight had four NNRTIs DRAMs, two participants had five NNRTI DRAMs. The maximal number of NNRTI DRAMs present in one participant was six, which was

found in three patients. It was further noted that there were 10 sequences which had NNRTI DRAMs but did not possess any NRTI DRAMs. Six participants had the rare NNRTI E138 series of DRAMs (E138K, E138A, E138Q and E138G) previously only reported in subtype B viruses that confer phenotypic resistance to ETR and RPV. All these participants had HIV-1 subtype A1. As a result, phenotypic drug resistance profiles against NNRTIs varied from susceptible, to potential low-level Resistance, to low-level resistance, to intermediate resistance to high level resistance in different proportions depending on the presence or absence of NNRTI DRAMs, the type and combination of DRAMs within the sequence. Of 140 samples that were successfully sequenced; 74, 62, 23 and nine demonstrated High-Level Resistance to NVP, EFV, RPV and ETR respectively (Figure 4.4). K103N present as the only NNRTI DRAM conferred high-level resistance to EFV and NVP but did not affect the ETR and RPV in phenotypic resistance profiles. K103E mutation did not confer any resistance to any of the NNRTI drugs. G109A mutation present alone conferred intermediate resistance to EFV, high-level resistance to NVP, potential low-level resistance to ETR and low-level resistance to RPV. Y181C mutation alone conferred intermediate resistance to EFV, ETR and RPV but high-level resistance to NVP while Y188L mutation in isolation conferred high-level resistance to EFV, NVP and RPV and potential low-level resistance to ETR. On the other hand, E138A; a very rare mutation in this population did not confer any resistance to EFV, NVP, but had effects of potential low-level resistance to ETR and low-level resistance to RPV when found in isolation whereas V179D mutation conferred high-level resistance to EFV and NVP as well as potential low-level resistance to both ETR and RPV.

Six participants reported the rare E138 series mutations (E138K, E138A, E138Q and E138G) that confer phenotypic resistance to ETR and RPV, previously only reported in HIV-1 subtype B. Five of the participants with E138 mutation series were subtype A1 while one was CRF A1_F (Table 4.6). Notably, one participant (SEQ084) had E138A as the only HIV DRAM and therefore exhibited only ETR and RPV. It was further noted

that 61 samples demonstrated susceptibility to both EFV and NVP while 76 samples demonstrated susceptibility to both ETR and RPV.

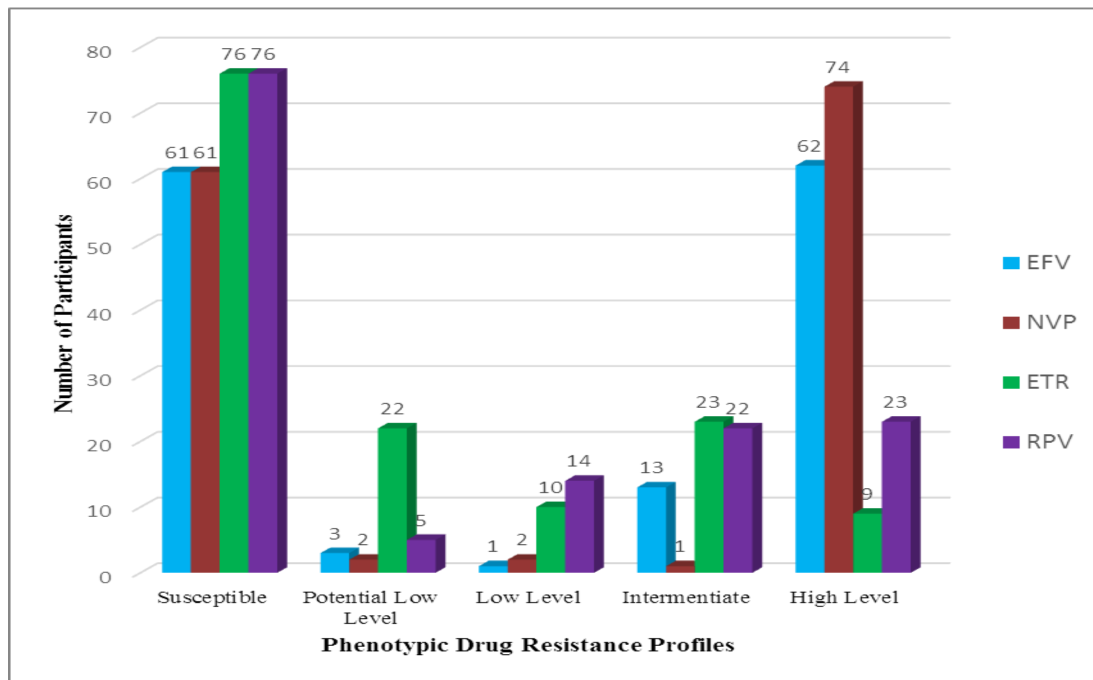


Figure 4.4: Number of phenotypic resistance profiles for different NNRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: “Susceptible” - no evidence of reduced ARV susceptibility compared with a wild-type virus. "Potential low-level resistance" – presence of mutations indicating previous ARV exposure or may contain mutation that are associated with drug resistance only when they occur with additional mutations. "Low-level resistance" - may have reduced ARV susceptibility. "Intermediate resistance" - high likelihood that a drug's activity will be reduced but that the drug will likely retain significant remaining antiviral activity. "High-level resistance" - patients infected with viruses having such mutations usually have little or no virological response to treatment with the ARV).

Subtype A1 represented 50%, subtype D represented 28.1%, subtypes A1_D and A1_C represented 4.7% , subtypes A1_J, B and G represented 3.1% whereas A1_F1 and B_C represented 1.6% of the participants with ETR and RPV resistance profiles.

Table 4.6: Summary of participants with E138 mutations among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

Sequence ID	Gender	Age	Viral Load (copies/ml)	Type of E138 Mutation	Regimen	Subtype
SEQ046	Male	67	546345	E138A	TDF+3TC+EFV	A1
SEQ076	Female	37	61436	E138G	TDF+3TC+EFV	A1
SEQ084	Female	28	6675	E138A	TDF+3TC+EFV	A1
SEQ106	Male	43	84453	E138Q	TDF+3TC+EFV	A1
SEQ112	Female	29	67186	E138K	AZT+3TC+NVP	A1_F1
SEQ131	Male	35	452839	E138Q	TDF+3TC+EFV	A1

4.3.3 Protease inhibitors (PI) DRAMs among patients receiving HAART in Busia County, Kenya.

A total of 12 different PI DRAMs conferred resistance to Atazanavir/r (ATV/r) and Lopinavir/r (LPV/r), the two main PIs in seven of the 140 participants who had drug resistance testing performed. M46I was present in three (25%), V82A/I/T and I84V were present in two (16.7%) of the patients that exhibited PI DRAMs while V32I, I47IM, N88ND, F53L and M46K were present in one (8.3%) patient exhibiting PI DRAMs in the cohort (**Table 4.7**). A combination of V32VI and I47IM PI mutations conferred low-level resistance to both ATV/r and LPV/r while N88D alone provided potential low-level resistance only to ATV/r. A combination of M46I, V82A/I/T and I84IV a conferred high-level resistance to all PI drugs as evidenced in the two patients with SEQ083 and SEQ109. Presence of F53L PI DRAM conferred potential low-level resistance to ATV/r while LPV/r remained susceptible. M64I PI DRAM alone conferred potential low-level resistance to ATV/r and LPV/r while M46K mutation in isolation did not confer resistance to any of the PI drugs. Only subtype A1 (85.7%) and subtype B (28.6%) contained PI DRAMs that conferred resistance to common PI drugs.

4.4 Drug resistance profiles among patients receiving HAART in Busia County, Kenya.

For NRTI category, drug resistance profiles for the different NRTIs ranged from susceptible to high-level resistance for the different NRTI drugs. High level resistance was present in 71 (50.7%) patients for FTC and 3TC, 11 AZT, and only three for TDF. A comparison between TDF and AZT revealed that both drugs had similar phenotypic resistance profiles overall whereas the phenotypic resistance profiles for FTC and 3TC were similar (Figure 4.5). Whereas there was no significant differences between the presence of the various drug resistance profiles among the NRTI drugs (p=0.3212), this study recorded significant differences were recorded between susceptible and potential low level resistance as well as between susceptible and low level resistance profiles for AZT and TDF (p=0.005).

Significant differences between susceptible and high level resistance was only recorded on TDF drug (p=0.00013). Overall, there were statistically significant differences in the presence drug resistance profiles across the NRTIs [susceptible, potential low level, low level, intermediate and high level] (p = 0.0008) with the difference in availability of these drug resistance profiles among the drug classes being insignificant (p=0.431). Of the 140 sequences analyzed, 107 (76.4%) demonstrated susceptibilities towards TDF, 102 (72.8%) and 67 (47.9%) towards both FTC and 3TC.

In the NNRTI category, phenotypic drug resistance profiles ranged from susceptible to high-level resistance for the different NNRTI drugs. Although NVP had higher high level resistance profiles than EFV, there were no significant differences between the resultant drug resistance profiles between these two drugs (p=0.4782).

Table 4.7: Summary of Protease Inhibitor (PI) HIV drug resistance associated mutations in patients treated with highly active antiretroviral therapy in Busia, Kenya.

ART Regimen		N/140	PI Mutations						
PI	NRTI		M46	V82	I84	V32	I47	N88	F53
Efavirenz (EFV) containing regimen									
EFV	TDF+3TC	9	3	1	1	1	1	1	1
	AZT+3TC	0	0	0	0	0	0	0	0
Nevirapine(NVP) containing regimen									
NVP	AZT+3TC	3	1	1	1	0	0	0	0
TOTAL		12	4	2	2	1	1	1	1

Interestingly, 79 participants demonstrated high, intermediate, low, potential low level resistance to EFV and NVP while sixty-one participants demonstrated susceptibility to both EFV and NVP (Figure 4.6). Seventy-six (76) participants demonstrated susceptibility to both ETR and RPV, while 22 and five participants had potential low-level resistance to ETR and RPV respectively. Ten patients demonstrated low level resistance to ETR while 14 participants had low level resistance to RPV. Twenty-three (23) patients demonstrated intermediate resistance to ETR with 22 participants demonstrating intermediate resistance towards RPV. There were nine participants with high level resistance to ETR compared to 23 participants with high level resistance to RPV. While comparing the two second generation NNRTIs (ETR and RPV), significant differences were reported in the presence susceptible vs potential low level (p=0.001), susceptible vs low level (p=0.0005), susceptible vs intermediate (p=0.035) susceptible vs high level (p=0.001) drug resistance profiles. .Overall, the differences between ETR and RPV drug resistance profiles were statistically significant (p=0.0015).

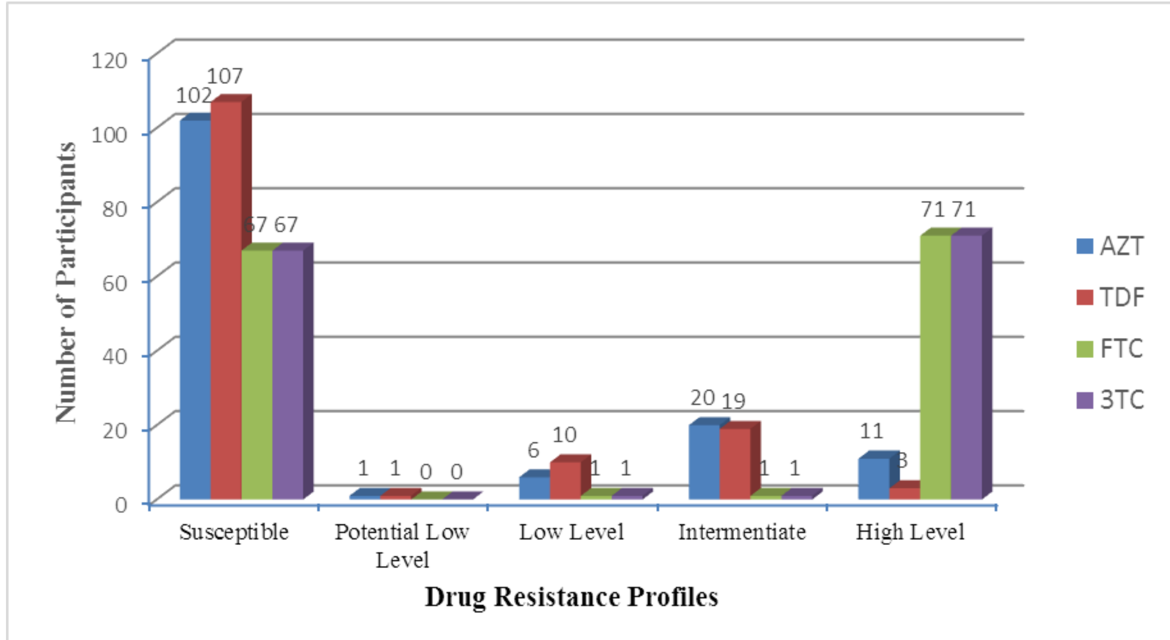


Figure 4.5: Distribution of the drug resistance profiles for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: "Susceptible" - no evidence of reduced ARV susceptibility compared with a wild-type virus. "Potential low-level resistance" – presence of mutations indicating previous ARV exposure or may contain mutation that are associated with drug resistance only when they occur with additional mutations. "Low-level resistance" - may have reduced ARV susceptibility. "Intermediate resistance" - high likelihood that a drug's activity will be reduced but that the drug will likely retain significant remaining antiviral activity. "High-level resistance" - patients infected with viruses having such mutations usually have little or no virological response to treatment with the ARV).

Similarly, ATV/r and LPV/r, the two available PIs exhibited similar phenotypic drug resistance profiles.

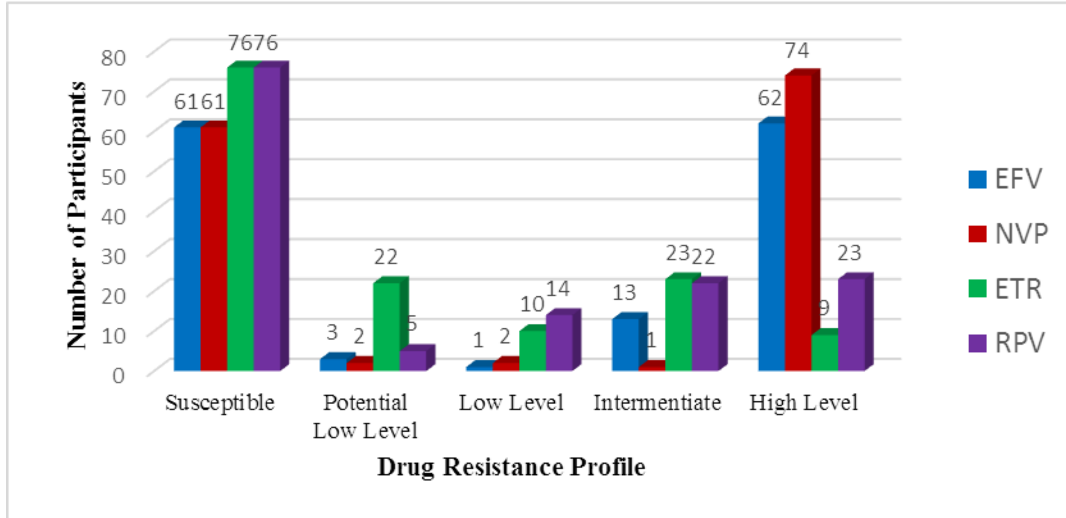


Figure 4.6: Distribution of the phenotypic drug resistance profiles for different NNRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: “Susceptible” - no evidence of reduced ARV susceptibility compared with a wild-type virus. "Potential low-level resistance" – presence of mutations indicating previous ARV exposure or may contain mutation that are associated with drug resistance only when they occur with additional mutations. "Low-level resistance" - may have reduced ARV susceptibility. "Intermediate resistance" - high likelihood that a drug's activity will be reduced but that the drug will likely retain significant remaining antiviral activity. "High-level resistance" - patients infected with viruses having such mutations usually have little or no virological response to treatment with the ARV).

4.5 HIV drug resistance scores among patients receiving HAART in Busia County, Kenya.

From the Stanford University HIV resistance database, the HIV drug resistance scores for both NRTI and NNRTI were obtained and downloaded to excel spread sheet for analysis. For NRTIs, AZT had 42 participants with negative scores (implying availability of mutations that make the virus more susceptible to drugs). For the participants with positive AZT scores, these ranged from zero to 145. FTC and 3TC did

not have any participant with a negative score, with the positive score ranging from 0 to 95 whereas TDF had negative scores and positive scores ranging from 0 to 65. For NNRTIs, EFV had all positive scores ranging from 0 to 165, NVP had positive scores ranging from 0 to 220, ETR had all positive scores to 100 whereas RPV had all positive scores ranging from 0 to 150. ATV/r scores ranged between 0-110 while LPV/r drug resistance scores ranged between 0-80. The two PIs did not exhibit any negative drug scores.

4.6 HIV subtype variation among patients receiving HAART in Busia County, Kenya

HIV Subtypes A1, A2, B, C, D, G, circulating recombinant forms (CRFs) A1_D, A1_C, A1_J, B_C, A2_H and A1_F1 were observed within this study population. Subtype A1 was the most prevalent subtype in the population, present in 75 (52.9 %) of the participants. Subtype D was present in 29 (20.7%), CRF A1_D was present in 10 (7.1%) of the population, subtype C and subtype B were both present in 6 (4.3%), of the population while subtype A2 was present in five (3.6%) of the population. CRF A1_C was present in four (2.9%) of the study population. Subtypes G was present in two (1.4%), whereas CRF B_C, CRF A1_F1 and CRF A1_J were all present in one (0.7%) of the population. CRFs accounted for 13.6% of the circulating HIV subtypes with majority of these CRFs (~79%) containing subtype A1. For this study, there were significant differences between the subtypes in relation to acquisition of DRAMs ($p = 0.0037$) as well as significant differences in presence of CRFs between males and females ($p=0.001$). Although discordance was reported between subtypes outcomes from REGA, Los Alamos National Library (lanl) and the Stanford University HIVdb subtyping software, these differences were not statistically significant ($p=0.4747$). There were subtype discordances between REGA subtyping tool, Los Alamos National Library (lanl) and the Stanford University HIVdb subtyping software in reporting all other subtypes except subtypes G, C and D. Interestingly, both lanl and the Stanford University HIVdb subtyping software did not report any CRF A1_D (Table 4.8). Only the lanl subtyping reported CRF A1B_C, CRF A1_B and CRF 01_AE.

While comparing the presence of DRAMs among the different subtypes, it was noted that out of the 53 patients who did not possess any DRAMs hence no phenotypic resistance to any of the available medications, 32(60.4%) were subtype A1, seven (13.2%) were subtype D, six were CRF A1_D, six (11.3%) were subtype C, while three (5.7%) were subtype A2. Of the 87 participants with at least one DRAM, 42 (48.3%) were subtype A1, 21 (39.6%) were subtype D, 20 (23%) were CRFs, 5(5.7%) were subtype A2, 5(5.7%) were subtype C while 4 (4.6%) were subtype A2 (Figure 4.**7**).

Of the 13 sequences with K65R mutations, 10 (76.9%) were subtype A1 while 3 (23.1%) were subtype D. Of the 37 sequences with at-least one TAM, 23 (62.2%) were subtype A1 or subtype A1 containing CRFs, 9 (24.3%) were subtype D or subtype D containing CRFs while 4 (10.8%) were subtype B. Participant gender significantly affected the HIV subtype present in an individual with female participants recording significantly higher proportions of subtype A1, D, A1_D, G and A2 (p=0.0006) while male participants had higher prevalence of subtype B and C (Figure 4.8).

Participant gender significantly affected the HIV subtype present in an individual with female participants recording significantly higher proportions of subtype A1, D, A1_D, G and A2 (p=0.0006) while male participants had higher prevalence of subtype B and C (Figure 4.8). It is important to note that no subtype C sequence had any DRAMs hence no drug resistance (meaning that all subtype C viruses were susceptible to all drug classes), whereas all subtype B sequences had DRAMs conferring resistance. Among the 19 participants with CRFs, only six did not contain any HIV DRAMs, 14 (73.6%) were females and majority (89.4%) were on EFV containing regimen (Table 4.9).

Table 4.8: Distribution of HIV subtypes circulating among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

HIV Subtype	REGA (%)	LANL (%)	Stanford (%)	Participants with DRAMS (%)
Subtype A1	74 (52.9)	86 (61.4)	86 (61.4)	43(58.1)
Subtype D	29 (20.7)	29 (20.7)	25 (17.9)	22(75.9)
CRF A1_D	10 (7.1)	0	0	4(40)
Subtype C	6 (4.3)	7 (5)	7 (5)	0(0)
Subtype B	6 (4.3)	12 (8.6)	14 (10)	6(100)
Subtype A2	4 (2.9)	0	4(2.9)	2(50)
CRF C_A1	4 (2.9)	0	0	4(100)
Subtype G	2 (1.4)	2 (1.4)	2 (1.4)	2(100)
CRF A1_J	2 (1.4)	0	0	2(100)
CRF C_B	1 (0.7)	0	0	1(100)
CRF A1_F1	1 (0.7)	0	0	1(100)
CRF A2_H	1 (0.7)	0	0	1(100)
Subtype A6	0	1 (0.7)	0	0
CRF A1_B_C	0	1 (0.7)	0	0
CRF A1_B	0	1 (0.7)	0	0
CRF 01_AE	0	1 (0.7)	0	0
CRF10_CD	0	0	2(1.4)	0
Total	140 (100)	140 (100)	140(100)	87

Table 4.9: Summary of demographic characteristics and DRAM profiles for sequences with CRFs among patients receiving highly active antiretroviral therapy in Busia, Kenya.

Sequence ID	Gender	Age	Viral Load (Copies/ml)	NRTI DRAMs	NNRTI DRAMs	Regimen	REGA Subtype
SEQ001	Female	25	120000	M41L, D67N, T69D, M184V, L210W, T215Y	K101E, Y181C, G190A	AZT+3TC+EFV	A1_D
SEQ015	Female	24	19875	NONE	K103N	TDF+3TC+EFV	A1_C
SEQ029	Female	42	32343	M184V, T215F	K101E, G190A	TDF+3TC+EFV	A1_C
SEQ031	Male	34	34435	M184V	K101KE, K103KN, G190S	TDF+3TC+EFV	A1_C
SEQ036	Female	24	23577	NONE	NONE	AZT+3TC+NVP	A1_D
SEQ038	Female	49	21233	M184V	A98G, G190A	TDF+3TC+EFV	A1_D
SEQ041	Female	32	186098	L74LV, M184V	K103N, P225H, K238T	TDF+3TC+EFV	A2_H
SEQ045	Female	32	34202	M184MIV	K103KN, G190GA	AZT+3TC+EFV	A1_J
SEQ047	Male	54	23543	M184V	K101KE, K103KN, G190S	TDF+3TC+EFV	A1_C
SEQ049	Female	43	54456	M184MV, T215TFIS	K101E, G190GA	TDF+3TC+EFV	B_C
SEQ053	Male	38	1504	NONE	NONE	TDF+3TC+EFV	A1_D
SEQ068	Male	54	9807	NONE	V108VI, G190GR	AZT+3TC+EFV	A1_D
SEQ090	Male	26	97070	M41L, L74I, M184V, T215F	K103N, V108VI, Y181YH, G190G, H221HY, K238KT	TDF+3TC+EFV	A1_J
SEQ093	Female	30	7059	NONE	NONE	TDF+3TC+EFV	A1_D
SEQ097	Female	29	6856	NONE	NONE	TDF+3TC+EFV	A1_D
SEQ101	Female	53	35958	NONE	NONE	TDF+3TC+EFV	A1_D
SEQ112	Female	29	67186	NONE	E138K, V179T	AZT+3TC+NVP	A1_F1
SEQ114	Female	38	84687	M184V	K103N, Y181C	TDF+3TC+EFV	A1_D
SEQ128	Female	35	3342	NONE	NONE	TDF+3TC+EFV	A1_D

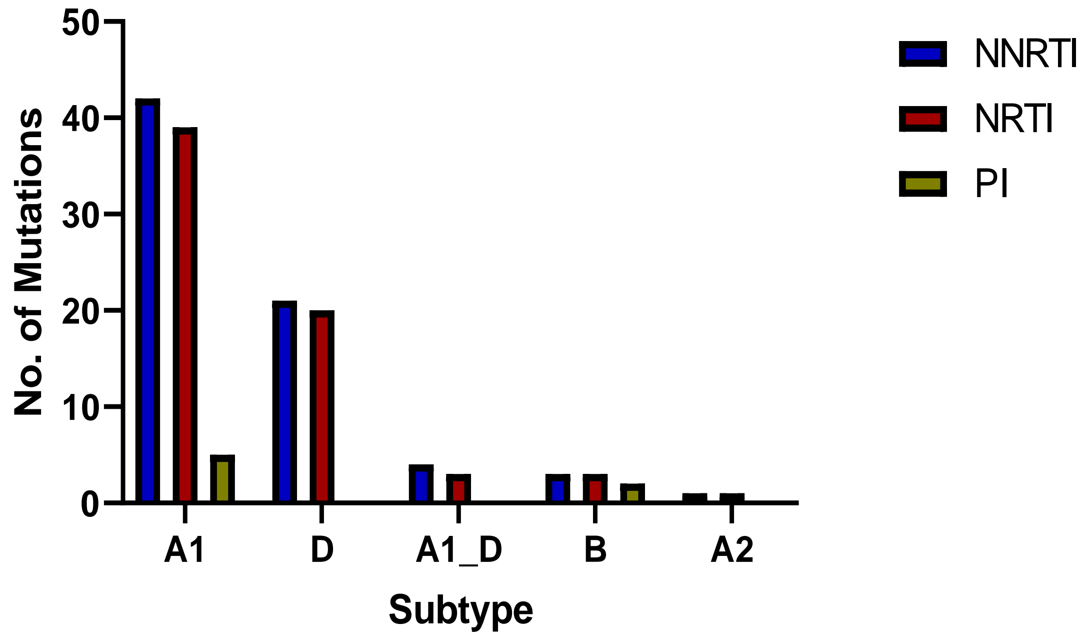


Figure 4.7: Distribution of different DRAMs among subtypes for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

4.7 Phylogenetic analysis among patients receiving HAART in Busia County, Kenya.

A total of one hundred and forty (140) samples were successfully sequenced and their sequences were analysed phylogenetically using Molecular Evolutionary Genetics Analysis (MEGA) phylogenetic analysis software version 11.0 (Kumar *et al.,* 2016).

HIV subtype A1, the most common subtype within this population and majority of its CRFs clustered with reference sequences from Kenya and Uganda with 0.701 bootstrap values lower in the phylogenetic tree. From the results, there were 12 distinct phylogenetic clusters HIV subtype A1. HIV-1 subtype A2 clustered with A2 reference sequences from Kenya with 0.6 bootstrap values.

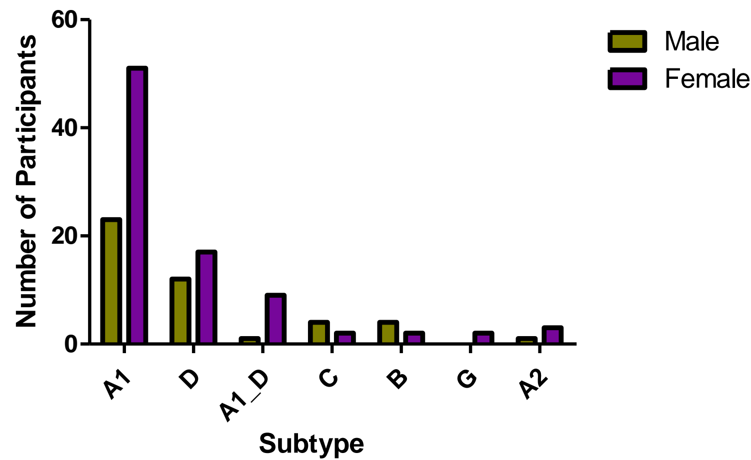


Figure 4.8: Distribution of subtypes among male and female participants for different NRTI drugs among patients receiving highly active antiretroviral therapy in Busia County, Kenya.

Subtype A1 and A2 sequences clustered close together lower in the phylogenetic tree. HIV subtype C and its recombinants clustered with sequences from Zambia and India with 0.702 bootstrap values. Subtype G sequences clustered with HIV subtype G reference sequences from Cameroon, Kenya and Ghana at 0.79 bootstrap values. These two subtypes (C and G) clustered close together in the phylogenetic tree (Figure 4.9 and Figure 4.10). HIV subtype D and majority of A1_D circulating recombinant forms clustered with reference sequences from Democratic Republic of Congo and Uganda at 0.901 bootstrap values. HIV subtype B sequences clustered together with consensus sequences from France and Thailand higher on the phylogenetic tree at 0.967 bootstrap values. HIV subtype A1_C and A1_D clustered together between subtype D and subtype G (Figure 4.11). The overall mean pairwise distance (d) was 0.03 and the standard error (S.E) was 0.01. The overall sum of all branches was 8.7115. These overall mean distances were calculated using the Jukes-Cantor with bootstrap variance estimation method at 1000 bootstrap replications. The codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were

eliminated (complete deletion option). There were a total of 17 positions in the final dataset. Evolutionary analyses were conducted in MEGA Version 11.

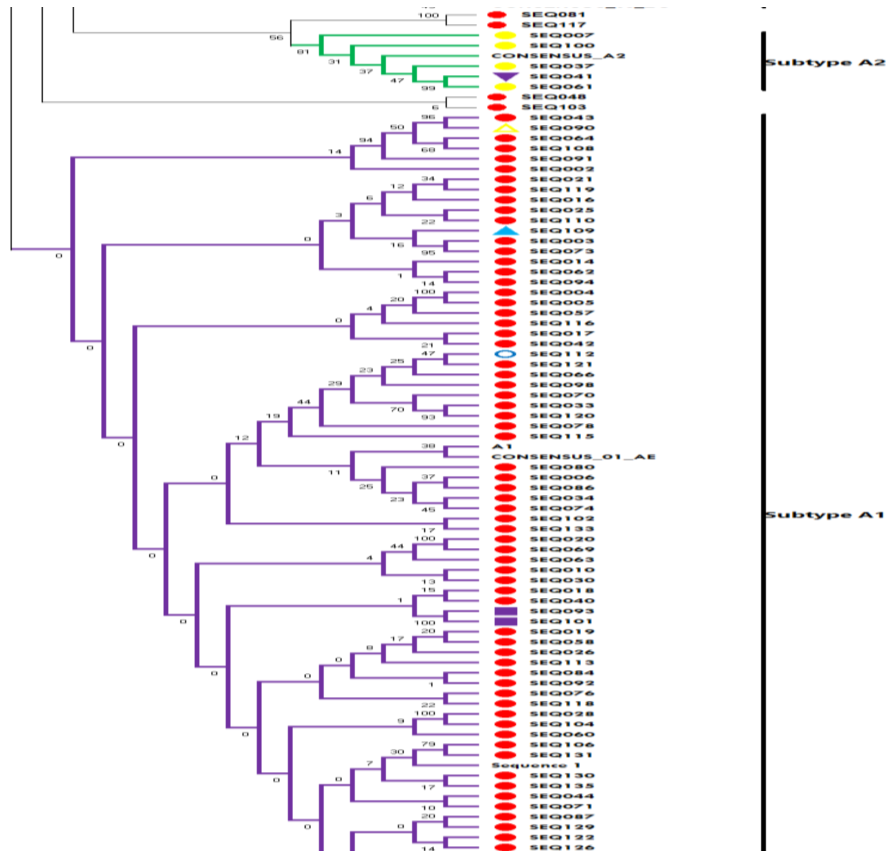


Figure 4.9: Phylogenetic tree of the HIV pol-RT gene showing HIV subtype A1 and HIV-1 subtype A2 among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: HIV-1 subtype A1 (Red filled circular labels), HIV-1 subtype A2 (Yellow filled circles), HIV-1 CRF A1_C (Purple filled square labels), subtype B (Blue filled triangular labels facing up), CRF A1_F1 (White filled circle labels with blue borders), CRF A1_J (White filled triangle labels with yellow borders), and CRF A2_H (Purple filled triangle facing down).

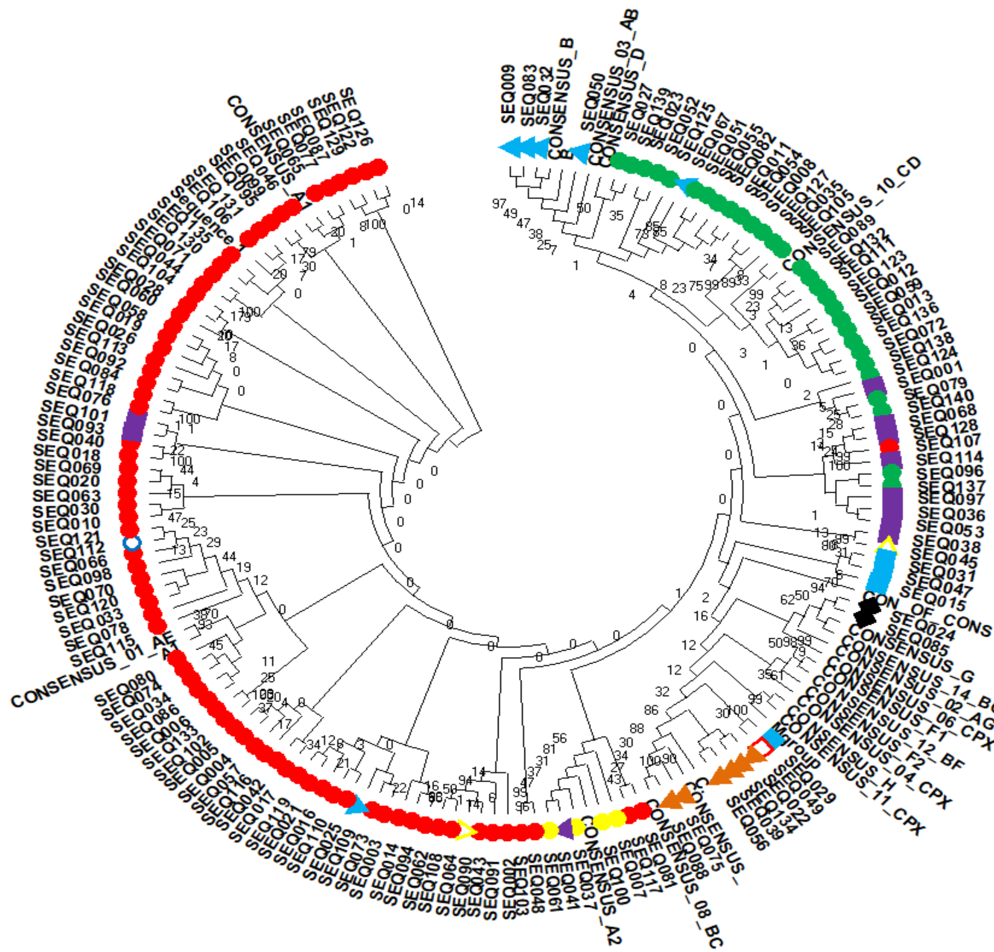


Figure 4.10: Circular phylogenetic tree of the HIV pol-RT gene among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: HIV-1 subtype A1 (Red filled circular labels), HIV-1 subtype A2 (Yellow filled circles), HIV-1 subtype C (Brown filled triangular labels facing down), HIV-1 subtype D (Green filled circular labels), HIV-1 subtype G (Black filled diamond labels), HIV-1 CRF A1_C (Purple filled square labels), CRF A1_D (Purple filled rectangular labels), subtype B (Blue filled triangular labels facing up), CRF A1_F1 (White filled circle labels with blue borders), CRF A1_J (White filled triangle labels with yellow borders), CRF A2_H (Purple filled triangle facing down) and B_C (White filled square with blue borders).

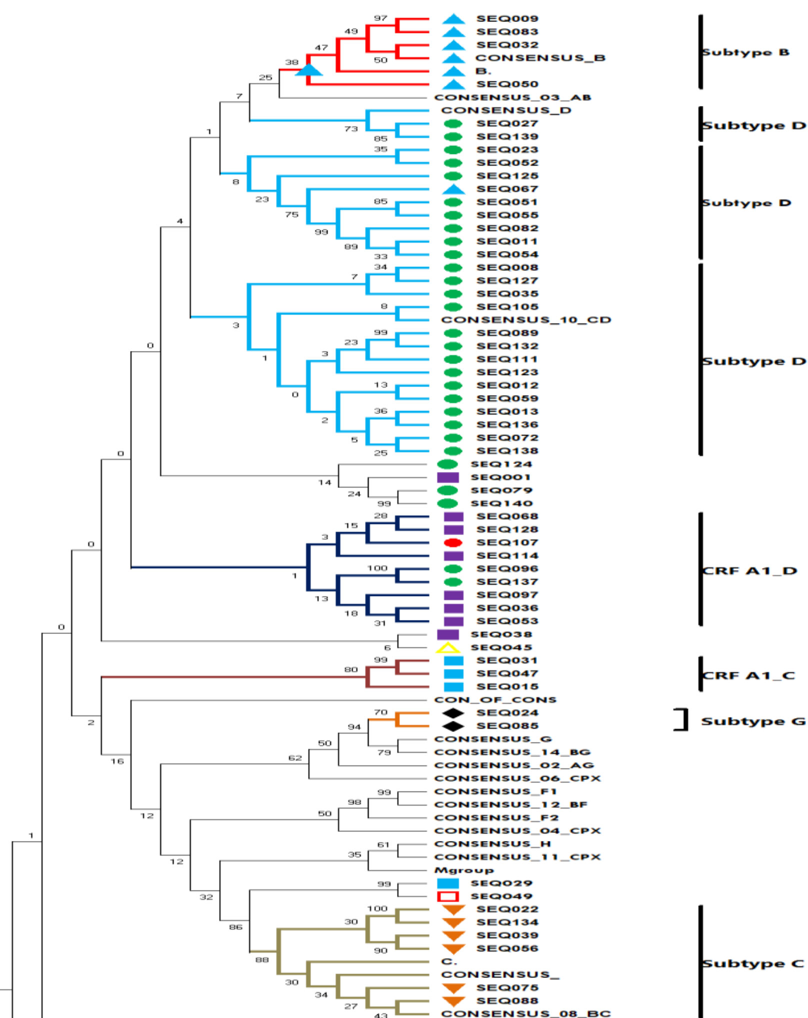


Figure 4.11: Phylogenetic tree of the HIV pol-RT gene showing HIV subtype A, HIV subtype D, HIV subtype A1_D, HIV subtype A1_C, HIV subtype G and HIV subtype C among patients receiving highly active antiretroviral therapy in Busia County, Kenya. (Key: HIV-1 subtype C (Brown filled triangular labels facing down), HIV-1 subtype D (Green filled circular labels), HIV-1 subtype G (Black filled diamond labels), HIV-1 CRF A1_C (Purple filled square labels), CRF A1_D (Purple filled rectangular labels), subtype B (Blue filled triangular labels facing up), CRF A1_J (White filled triangle labels with yellow borders), CRF A2_H (Purple filled triangle facing down) and B_C (White filled square with blue borders).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The main aim of this study was to evaluate markers of treatment failure, with special emphasis on virologic outcomes, HIV viral load counts and drug resistance after 12 months of standard antiretroviral therapy in a cross-sectional study in Busia County Referral Hospital, Western Kenya. The study specifically determined the level of HIV viral load counts, HIV drug resistance associated mutations in this population, described and ranked specific HIV-drug resistance (HIV-DR) mutations and resistance patterns among participants not achieving virologic suppression (virologic failure). Additionally, the study also determined if viral load and resistance testing combined may improve the management and antiretroviral therapeutic approach of patients enrolled in the programme based on the resistance patterns. The study further sought to determine the HIV subtypes circulating in the cohort, the phylogenetic distances of the different virus strains circulating in the cohort and comparing the utility of HIV drug resistance reports generated using the Stanford University drug resistance database (Appendix I). In addition, phylogenetic distances between the different subtypes were generated as well.

About 37.9% of patients showing treatment failure by viral load testing did not contain any DRAMs hence no HIVDR profiles to any ART drug classes reported. This means that if virologic failure was solely used alone to determine failure, 53/140 (37.9%) patients who did not arbor any DRAM and were susceptible to all the available drugs would have been switched to a second-line regimen. In addition, there were participants with virologic failure who had both genotypic and phenotypic resistance to NRTIs but were susceptible to NNRTIs and vice versa. It would take drug resistance testing to make sound treatment decisions for such cases. Additionally, some patients demonstrated resistance to PIs despite them being on first line regimens. Baseline drug resistance testing would have been crucial in identifying transmitted drug resistance.

5.1.1 HIV RNA viral load testing: a measure of HIV treatment failure among patients receiving HAART in Busia County, Kenya

There is sufficient scientific evidence to prove that viral load (VL) testing is a reliable measure of treatment failure (Brijkumar *et al.,* 2020; Mwau *et al.,* 2018) and that HIV transmission is significantly reduced by lowering viral loads (Bulage *et al.,* 2017; Cherutich *et al.,* 2016; Mwau *et al.,* 2018; Sandbulte *et al.,* 2020). This study tested for VL counts in all the eligible participants in the programme to determine the level of treatment failure within the population. The percentage of patients in this population taking ART but with detectable viral loads (>40 copies/ml) was 23.8% of the study population. The reported results of 23.8% detectable VL from the current study compared greatly to the results from the 2012 Kenya AIDS Indicator Survey (KAIS), a survey that evaluated the impact of ART coverage on viremia and examined the risks for failure to suppress population-level HIV VL suppression in Kenya which reported that 26.1% of persons taking ART in Kenya had detectable viremia (Cherutich *et al.,* 2016). Results on the detectable viral load from the current study also compared to those of Kantor *et al*. (2014) who reported a detectable viral load of 29% from a study looking at the diversity of HIV and HIVDR from plasma and non-plasma samples in a large treatment programme in Busia County Referral Hospital, Western Kenya. There was agreement between the results of the current study and those of a study on a population of HIV-positive children on ART in Uganda which reported ~23% non-suppression rates (Nabukeera *et al.,* 2021). This means that the rate of detectable viral load for patients on ART in the current study population compares to the rates previously reported in Uganda. The present study therefore contributes to the growing body of evidence that a good proportion (24-29%) of patients on ART still have detectable HIV viremia, a great concern for many HIV treatment and prevention programmes (Cherutich *et al.,* 2016; Dube & Stein, 2018; Kassaye *et al.,* 2019). ART provision to HIV infected individuals is aimed at reducing HIV viremia to undetectable levels thereby reducing its transmission as well as HIV related morbidity and mortality (Cherutich *et al.,* 2016; Günthard *et al.,* 2016). The presence of high percentages of detectable HIV viremia in

treatment programmes calls for concerted efforts from all stake holders to bring these levels down for treatment success, especially if the 95-95-95 HIV prevention strategy is to be realized (Kassaye *et al.,* 2019). The present study found significant relationship between both gender and age with detectable viral load counts (p = 0.0001), thus partially agreeing with results obtained by Cherutich *et al.,* (2016) who showed no significant difference between gender and detectable viremia (p = 0.308), but found a correlation between age and detectable viremia (p = 0.0001). The results of the present study disagreed with those reported by Bulage *et al.,* (2017) in a study on HIV-positive patients on ART in Uganda who noted that the odds of HIV non-suppression decreased with increase in age. Important to note is the fact that the study by Bulage *et al.,* (2017) had significantly higher number of participants (>100,000) compared to <1,000 participants for the current study which could have led to differences in the odds of non-suppression between the two studies. The present study reported an overall virological failure of 15.9% which was close but slightly higher than that observed by Bulage *et al.,* (2017) which reported an overall failure rate of 11%. Sustained virologic failure in patients on ART for more than 12 months in this study was therefore confirmed.

5.1.2 HIV DRAMs conferring resistance to ART among patients receiving HAART in Busia County, Kenya

This study recorded a high sequencing success rate of 95.8% and detected major DRAMs that conferred resistance against NRTI, NNRTI and PI drugs. Of the samples successfully analyzed, 63.5% had at least one major HIV DRAMs against the PIs, NNRTIs, NRTIs or a combination of two or more of the above that led to development of HIVDR against at least one class of antiretroviral drugs. Out of 140 successful sequences, 37.9% did not possess any DRAMs, hence did not show display HIVDR towards available antiretroviral drugs despite having virologic failure. This was in concordance with reports by Zhou *et al*. (2016) on high levels drug sensitivity with virologic failure. There is consistency in findings between this study and several other studies which have likewise reported high levels (~25%) of HIV drug sensitivity (susceptibility) despite with virologic failure (Dutta & Saha, 2018).

5.1.2.1 NRTI DRAMs among patients receiving HAART in Busia County, Kenya

Seventy-seven (77) out of 140 successful sequences (55%) had at least one or more NRTI DRAMs with M184V/I being present and the most prevalent in 50% of the 140 successful sequences. The predominance of M184V/I DRAMs agrees with several studies have previously reported the predominance of M184V/I NRTI DRAMs in different African settings and populations (Kantor *et al.,* 2014; Saravanan *et al.,* 2017; Waihenya *et al.,* 2015). For instance, the current study reported a lower prevalence of M184V/I compared to Waihenya *et al*. (2015) who reported a prevalence of 51.8% M184V NRTI DRAMs among patients failing first line ART IN Nairobi, Kenya. Kantor *et al*. (2014), observed a prevalence of 76% of M184V mutations. Other studies have reported even higher levels of M184 DRAMs in different populations. A study on ETR and RPV HIVDR in HIV subtype C infected children failing NRRTI-based regimens in South India reported that M184V/I HIV DRAMs were present in an estimated 94% of the children population (Saravanan *et al.,* 2017). Generally, this current study reported lower levels of M184V/I DRAMs than most of the published literature.

M184V/I DRAMs are low genetic barrier non-thymidine analogue NRTI mutations that are selected for by lamivudine and emtricitabine and when present, they reduce susceptibility to these drugs by 100-fold resulting in high level resistance to these drugs (Dube, 2018; Skhosana *et al.,* 2015). When the mutation also is selected for it leads to low-level HIVDR to abacavir and didanosine (Dube, 2018; Saravanan *et al.,* 2017; Skhosana *et al.,* 2015). M184V/I DRAM is among the few NRTI mutations that are capable of causing resistance to the target drugs even in the presence of a single primary drug mutation due to its low genetic barrier (Dube, 2018; Skhosana *et al.,* 2015). In the present study, 25 (17.6%) of the participants had M184V/I DRAMs as the only NRTI mutation. M184V/I mutation when present alone provided high level resistance to 3TC and FTC but had no notable impact on AZT and TDF. This finding is in concordance with many other studies that have reported that M184V/I increases susceptibility to AZT and TDF while conferring high-level HIVDR to 3TC and FTC (Hung *et al.,* 2019; Mouradjian *et al.,* 2020). The seven participants with M184I version of this DRAM with

majority possessing the M184V version implied that only a few of the study participants were in their initial stages of resistance with majority having advanced drug resistance as indicated in previous studies (Hung *et al.,* 2019).

T215F/Y/I/N is a type 2 thymidine analogue mutation (TAM) selected for by the thymidine analogs AZT and d4T. This mutation causes intermediate-level HIVDR to AZT and d4T as well as low-level HIVDR to ABC, ddI and TDF (Mouradjian *et al.,* 2020). As was the case of the current study, many other studies in the high HIV burdened countries have reported T215F/Y/I/N NRTI DRAMs as the second most prevalent mutations after the M184V/I (Ibe & Sugiura, 2011; Kantor *et al.,* 2014; Saravanan *et al.,* 2017). Similarly, results from the current study agree with those of Saravanan *et al.* (2017) who from their study on children population in India reported that T215F/Y/I/N (41%) NRTI DRAMs were second most prevalent DRAMs after M184V/I. As reported in the current study, similar results were obtained by Kantor *et al.* (2014). It was observed that in the present study, T215F/Y/I/S/N TAMs did not appear alone as NRTI DRAMs, but was always in combination with other NRTI DRAMs unlike the M184V/I DRAMs.

Among all the samples that were successfully sequenced, there was no case where DRAM K65R existed in the presence of any of type 1 or type 2 TAMs. This is in agreement with reports by Recordon-Pinson *et al. (*2018), who showed antagonism between K65R and T215F/Y/I/N TAMs. As described earlier, K65R is found in negative antagonism with the T215F/Y/I/N and other TAMs (Saravanan *et al.,* 2017). In the study, none of the 13 samples with K65R/Q possessed T215F/Y/I/N DRAMs. The study therefore confirmed antagonism between K65R and T215F/Y/I/N DRAMs as previously reported (Saravanan *et al.,* 2017). K65R mutations were found in combination with M184V/I DRAMs in 12/13 sequences with K65R mutations, confirming that M184V/I was mostly associated with the emergence of K65R mutations in the study population as demonstrated by Inzaule et al. (2016). Only one out of the 13 sequences with K65R had TAMs (D67N, K70R and K219Q) implying that K65R had a negative effect on the presence of TAMS. These results agreed with Recordon-Pinson *et al,* (2018), who

reported negative association between K65R and TAMs. Despite the low genetic barrier K65R mutations, these DRAMs are found in low numbers since viruses with this mutation have an impaired replicative capability and compromised viral fitness (Saravanan *et al.,* 2017). When both K65R and M184V/I mutations are present within the same virus, there is counter-selection (antagonism) between K65R and TAMs as well as the subtype dependence (with subtype A1 being reported to be more prone to K65R mutations) in K65R mutation development (Derache *et al.,* 2016).

Other TAMs observed in this population were M41L, K70R/N, D67N/Y, K219R/Q/E and L210W present in 9.2%, 8.7%, 7.6%, 6.0% and 2.2% respectively. Ibe & Sugiura, (2011), identified TAMs as the second most prevalent DRAMs after M184V/I as reported in this study. The K65R mutation selected for by TDF, ABC, d4T, ddI, and sometimes by 3TC (Inzaule *et al.,* 2016; Skhosana *et al.,* 2015). This mutation reduces TDF, ABC and ddI susceptibility by approximately 2-fold and d4T susceptibility ~1.5-fold while increasing the susceptibility of AZT (Derache *et al.,* 2016; Skhosana *et al.,* 2015; Sunpath *et al.,* 2012). This was confirmed in the present study as majority of patients with K65R mutations harbored viruses susceptible to AZT and phenotypic resistance profiles to all other NRTIs.

Potential low level to high- level resistance to different NRTIs has previously been associated with M184V/I, K65R and the different TAMs in many settings (Takou *et al.,* 2019). This was clearly confirmed in this study where these mutations contributed to different levels of resistance to AZT, 3TC, FTC and TDF. Higher levels of resistance have been reported in 3TC and FTC than in both TDF and AZT (Derache *et al.,* 2016; Takou *et al.,* 2019). This was demonstrated in this study where ~52% of all patients had resistance to both FTC and 3TC in equal measure compared to 27% and 23.6% resistance to AZT and TDF respectively. On the other hand, TDF has been shown to be less prone to resistance compared to AZT (Derache *et al.,* 2016) which was in concordance with the findings of this study which reported 27% and 23.6% resistance levels for AZT and TDF respectively. Coupled with low adherence due to a higher pill burden, toxicity, higher frequency of failure of AZT compared to TDF, TDF containing

regimens are now preferred in most parts of the world hence many treatment programs have transitioned to TDF containing regimens (Derache *et al.,* 2016; Gibson *et al.,* 2017). A high degree of cross resistance between AZT and TDF as well as between FTC and 3TC in were documented in the present study as previously reported (Derache *et al.,* 2016; Gibson *et al.,* 2017).

5.1.2.2 NNRTI DRAMs among patients receiving HAART in Busia County, Kenya

NNRTI DRAMs usually confer resistance to and are indicated by EFV, NVP, RPV and ETR which are the main NNRTI drugs used in many parts of the globe as treatment against HIV (Saravanan *et al.,* 2017). EFV and NVP are the most commonly used NNRTI drugs in both the developed world and the resource limited settings like Kenya (Siedner *et al.,* 2020; Koigi *et al.,* 2014). In most sub-Saharan African countries and other resource limited settings where the HIV burden is highest, EFV is the available first-generation NNRTIs with NVP being most commonly used for prevention of mother to child transmission (Koigi *et al.,* 2014; Saravanan *et al.,* 2017). This was observed the demographics of the current study since majority of the participants were on an EFV based regimen and only female participants were on NVP containing regimens. The use of both NVP and EFV as first generation first line NNRTIs is limited by their low genetic barrier to resistance (meaning that a single-nucleotide change has the potential of leading to high-level HIVDR with little while still maintaining the replication capability of the virus optimized) and notable cross resistance between these two drugs (Saravanan *et al.,* 2017) . Evidently, there was a high degree of cross resistance between NVP and EFV as well as between ETR and RPV. Notably, K103N/S family of DRAMs was the most prevalent NNRTI DRAM followed by G190A/S, K101E/P, H221Y, Y181C/S/H, V108I and K238T respectively. The current study reported K103N/S/E as the most prevalent NNRTI DRAM, which concurs with many studies with similar findings (Zuo *et al.,* 2020; Saravanan *et al.,* 2017; Zhou *et al.,* 2016; Waihenya *et al.,* 2015). Despite the low genetic barrier of K103N/S, the mutation is found in relatively low numbers since its presence lowers the fitness of the virus (Zuo *et al.,* 2020). Eight patients who had the K103N NNRTI mutation alone recorded high level resistance to EFV and NVP

as previously reported in literature (Waihenya *et al.,* 2015). The prevalence of K103N/S DRAMs (19.8%) in the present study was lower than that reported by Kantor *et al.,* (2014) who reported a prevalence rate of 40%. Dow *et al*. (2014) also reported a prevalence of 40% of K103N/S in a study of perinatally infected children in Tanzania, a prevalence that was higher than what was reported in this current study. Wilhelmson *et al*. (2018), reported predominance of pre-treatment K103N/S DRAMs in treatment naïve patients in Guinea Bissau, an indication that this K103N/S/E is an important mutation in both treatment of naïve and experienced patients (Wang *et al.,* 2014). K103N/S/E family of DRAMs are NNRTI DRAMs selected for by NVP and EFV, the two main drugs used for first line treatment in sub-Saharan Africa and other high burden HIV regions whose presence reduces the susceptibility of EFV, NVP, ETR and RPV (Moyo *et al.,* 2020; Wang *et al.,* 2014). K103N + L100I, K103N + V108I and K103N + P225H double mutants were all found in the same ratio among the patients in the study population and all conferred high level resistance to EFV and NVP, contrary to previous reports where K103N + L100I combination was comparatively found in lower proportions (Moyo *et al.,* 2020). G190A/S/R DRAMs were mostly found in association with other NNRTI DRAMs but was rarely associated with K103N/S/E mutations indicating a possibility of competition between the two sets of mutations as reported by Wang *et al*. (2014). In the present study, K101E/P and H221Y had higher prevalence than the Y181C/S/H DRAM most likely due to the higher replicative competency of the mutant viruses, a phenomenon which has not been reported in many studies (Moyo *et al.,* 2020; Wang *et al.,* 2014).

The possible second generation NNRTIs after failure of NVP and EFV are ETR and RPV with ETR being more preferred due to its high genetic carrier to resistance (Bissio *et al.,* 2017; Saravanan *et al.,* 2017). The development of cross resistance between RPV and ETR in HIV-seropositive patients taking EFV and NVP provides a major setback in the use of ETR and RPV as second generation NNRTIs (Bissio *et al.,* 2017; Diphoko *et al.,* 2018). From this study, it was noted that 64 (45.7%) of all sequences had ETR and RPV HIVDR profiles ranging from potential low level HIVDR to high level HIVDR.

ETR and RPV resistance mutation profiles did not occur in isolation in this study, meaning that all the patients who exhibited resistance to ETR also showed resistance to RPV. It was however noted that RPV had higher resistance profiles than ETR implying higher genetic barrier for ETR compared to RPV as reported previously (Diphoko *et al.,* 2018).

Among the patients with ETR and RPV HIVDR, the main ETR and RPV DRAMs in this study were K103N/S (39%), K101E (29.7%), Y181C (26.5%), E138A/K/Q (9.4%), K238T (17.2%), V179D/T/E (6.3%) and L100I+K103N (4.7%). These results agreed with results from many other studies that have previously reported K101E, Y181C and E138A/K/Q as major ETR and RPV mutations (Diphoko *et al.,* 2018).

It was further noted that 57 (89.1%) of all the 64 participants harboring second-generation NNRTI resistance had M184/I mutations. This presents high prevalence coexistence of M184V/I mutations with ETR and RPV DRAMs as reported by (Bissio *et al.,* 2017; Diphoko *et al.,* 2018). As reported earlier, this study identified a cluster of 13 patients who had either NNRTI or NRTI DRAMs in isolation. It was noted that there was a greater probability of occurrence of isolated NNRTI DRAMs without the presence of NRTI or PI DRAMs. This study noted double the number sequences with NNRTI DRAMs that did not contain any NRTI DRAM (10 for NNRTI and 5 for NRTI) than the vice versa.

The current study concurs that K103N, G190A, K101E/P, H221Y, Y181C/S/H, V108I and K238T among other minor NNRTI DRAMs in isolation or in combination have been shown to cause varying degrees of phenotypic resistance to NNRTIs (Zhou *et al.,* 2016). Low to high level NNRTI resistance was witnessed in this study resulting from one or more NNRTI DRAMs as previously reported in literature. Numerous studies have demonstrated almost similar rate of phenotypic resistance in NVP compared to EFV (Bissio *et al.,* 2017; Diphoko *et al.,* 2018; Zhou *et al.,* 2016). On the contrary, the present study showed lower differences between NVP and EFV phenotypic resistance. The present study confirmed higher susceptibility profiles to second generation NNRTIs

compared to primary NNRTIs as demonstrated in many previous studies (Zhou *et al.,* 2016). There are conflicting reports on the similarity between ETR and RPV HIVDR profiles. The results of the current study contradict reports by Saravanan *et al*. (2017), reported 65% and 47% RPV and ETR HIVDR profiles respectively. Teeranaipong *et al*. (2014) on the other hand reported 31.6% and 32.2% susceptibility for RPV and ETR respectively, thereby agreeing with the results from this study.

5.1.2.3 Protease inhibitors (PI) HIVDR related mutations

According to the inclusion criteria, all the participants were on 1st line ART regimen which includes two NRTI and one NNRTI drugs as per the guidelines at the time of the study (Budambula *et al.,* 2015; Ministry of Health & National AIDS and STI Control Programme, 2016; Waihenya *et al.,* 2015). Seven participants in this study had PI DRAMs despite being naïve to PI drugs. This could be an indicator of potentially transmitted drug resistance as previously reported (Budambula *et al.,* 2015). The emergence of this transmitted HIVDR has been and will continue to be one of the major drawbacks towards the 90:90:90 HIV prevention strategies (Budambula *et al.,* 2015; Phillips *et al.,* 2017). These results agree with other studies on that reported presence of PI DRAMs in ART naïve patients (Budambula *et al.,* 2015; Phillips *et al.,* 2017).

5.1.3 HIV subtype variation among patients receiving HAART in Busia County, Kenya

From this study, high diversity of HIV subtypes circulating in Busia was confirmed with subtype A1 being the most predominant (52.9%). Several other studies have similarly reported the predominance of HIV subtype A1 in majority of the Kenyan HIV seropositive populations (Gounder *et al.,* 2017; Waihenya *et al.,* 2015; Adungo *et al.,* 2014). The current study reported slightly higher prevalence of subtype A1 compared to the study by Gounder *et al.* (2017), also reported subtype A1 to be the predominant subtype (44.4%). According to the results of this study, subtype D recorded the second highest prevalence in the population (20.7%), disagreeing with the findings by Gounder

et al. (2017), who reported subtype C to have the second highest prevalence but agreeing with results from studies by Kantor *et al*. (2014), and Lihana *et al.* (2009).

Results from the current study agreed with those by Oyaro *et al.,* (2011) who reported comparable HIV subtype A1 prevalence of 51.4% which is comparable to 52.8% in the present study but a considerably lower subtype D prevalence (9.4% vs 22.6% for this study). While this study reported a slightly lower subtype D prevalence (22.6%) than the study by Adungo *et al.* (2014), (28%), there were comparatively higher prevalence of CRF A1_D in the current study (7.1%) compared to 1.3% for the Adungo study. This study further agrees with several other studies on the predominance of HIV subtype A1 in Western Kenya, with an increasing HIV subtype D and CRF A1_D within the population in Busia over the years (Adungo *et al.,* 2014; Makwaga *et al.,* 2020). Overall, the present study reported higher levels of subtype D compared to majority of the previous studies (Gounder *et al.,* 2017; Waihenya *et al.,* 2015; Adungo *et al.,* 2014).

The current study reported lower prevalence of subtype A1 compared to the study by Lel *et al*. (2014), who reported prevalence of 69.8% for HIV subtype A1 but a similar prevalence of HIV subtype D (22.6%). Importantly, Lel *et al*. (2014) did not report any CRF A1_D. Important to note is the fact that their study examined transmitted HIV subtypes in infants whereas the current study looked at HIV subtypes in adults hence the possible explanation for the differing prevalence in the circulating subtypes.
Hassan *et al*. (2013), in a study on transmitted HIVDR in a rural settings in Kilifi, Kenya, reported a prevalence of 52%, 12% and 4.4% for subtypes A1, D and C respectively. Evidently, there is an elevated subtype D prevalence in the current study compared to the study by Hassan *et al.,* (2013). Additionally, the current study reported relatively lower CRF prevalence of 13.6% compared to the CRF prevalence of 31% reported by Hassan *et al*. (2013). The considerably lower rates of CRFs could be indicative of lower rates of multiple infections in this current study population. The differences in prevalence could also be attributed to the fact that unlike the Kilifi study which evaluated transmitted drug resistance, the present study examined induced drug resistance.

The HIV subtype in an individual plays a major role in development of HIVDR towards ART (Gartner *et al.,* 2020; Santoro & Perno, 2013), viral transmission rates (Gartner *et al.,* 2020; Macharia *et al.,* 2020; Santoro & Perno, 2013) and disease progression rates (Gartner *et al.,* 2020), with subtype D showing faster progression to disease and higher mortality rates than subtype A1 (Gartner *et al.,* 2020; Santoro & Perno, 2013; Ssemwanga *et al.,* 2013). Several studies have demonstrated that HIV subtype D and C are more vulnerable to development of drug resistance than subtype A (Gartner *et al.,* 2020; Santoro & Perno, 2013). This study supports the findings from previous studies on the vulnerability of subtype D to HIVDR development since 75.9% of subtype D sequences had DRAMs compared to 53.4% subtype A1 sequences. All the subtype C samples in the present study were susceptible to all classes of drugs, in contrast with findings by Santoro & Perno, (2013) who recorded higher HIVDR in subtype C than any other subtype. Several studies have reported higher rates of HIV mother to child transmission in mothers having subtype C and D than in those with subtypes A, B and their CRFs (Office of AIDS Research Advisory Council, 2019; Kapaata *et al.,* 2013). Patients with HIV subtype D have also been reported to progress faster to disease and have lower rates of transmission than those with subtype A viruses which are majorly dual tropic (Ssemwanga *et al.,* 2013). Subtype A has been shown to have higher viral transmission rates than subtype D (Koigi *et al.,* 2014). Data from the present study supports previous that imply high prevalence of subtype A1, D and CRF A1_D in populations may pose a challenge to HIV treatment and prevention strategies.

The present study detected a higher prevalence of subtype D (22.6%) and CRF A1_D (7%) compared to most other studies in Kenya (Gounder *et al.,* 2017; Kantor *et al.,* 2014; Koigi *et al.,* 2014). HIV subtype D has been reported to be more prevalent in Uganda (Kapaata *et al.,* 2013). A higher prevalence of subtype D in this population could therefore be indicative of cross border infections as the study site borders Uganda. Continuous monitoring of the HIV subtypes circulating within the population is therefore key in formulating strategies aimed at reducing the prevalence of HIV, reducing the rates of HIV transmission as well as taming AIDS related morbidity and mortality.

5.1.4 HIVDR resistance profiles, scores and phylogeny among patients receiving HAART in Busia County, Kenya

Among the NRTI drug family, FTC and 3TC have higher drug resistance patterns compared to AZT and TDF. This is because the M184V/I DRAM (the most prevalent NRTI mutation) greatly reduces 3TC and FTC susceptibility (Phillips *et al.,* 2017) while increasing the susceptibility and slowing down the emergence of resistance of AZT and TDF. Similarly, significantly higher phenotypic resistance levels for 3TC and FTC as compared to AZT and TDF were recorded as previously reported (Gibson *et al.,* 2017).

TDF remains a preferred drug of choice since AZT is comparatively more expensive, it requires a twice daily prescription that is tedious to the patient and may affect adherence, has long-term negative side effects such as anemia, neutropenia, hepatotoxicity and the faster rate at which HIVDRAMs against AZT develop on exposure compared to TDF (Gibson *et al.,* 2017). The similarity in the number of patients with phenotypic resistance towards EFV and NVP was an indicator of the high levels of cross-resistance between the two drugs as previously reported (Sluis-Cremer, 2014). The present study clearly demonstrated alarmingly high levels of cross resistance between EFV and NVP and similar levels between RPV and ETR which has been demonstrated to be as a result of the presence of nearly all NNRTI DRAMs within or near the NNRTI drug binding pocket and the low genetic barrier to NNRTI HIVDR with EFV, NVP and RPV requiring only one DRAM to develop resistance (Sluis-Cremer, 2014). Despite the slight differences in the number of patients within the specific level of HIVDR profiles between RPV and ETR, their levels of HIVDR profiles were similar. This is a demonstration of potentially high levels of cross-resistance between these two second generation NNRTI drugs. It is however worth noting that patients with high level HIVDR against RPV were twice as many as those with high level HIVDR to ETR. The statistically significant high level HIVDR of RPV compared to ETR presents ETR as the preferable drug of choice in case of NVP and EFV failure.

Among the seven drugs studied, only AZT and TDF had negative drug scores. This is associated with mutations such as M184V and K65R that selectively increase susceptibility to these drugs while reducing likelihood of emergence drug resistance (Skhosana *et al.,* 2015; Sluis-Cremer, 2014; World Health Organization, 2017). Indeed, the low M184V genetic barrier allows this mutation to develop rapidly in 50% of the patients under NRTI treatment and is advantageous to the patient since it results in lower viral replicative fitness, antagonizes development of K65R mutation and reduces resistance to NNRTIs and PIs (World Health Organization, 2017). NVP and EFV had the highest resistance scores of 220 and 165 respectively which was indicative of their low genetic barriers to resistance among NNRTIs as previously reported (Sluis-Cremer, 2014; World Health Organization, 2017).

HIV is a highly diverse virus with mixed subtypes and CRFs present in different populations and geographic locations around the globe (Nduva *et al.,* 2020). HIV exhibits several mechanisms within the viral structure that contribute to rapid viral evolution giving rise to the many different subtypes and CRFs (Nduva *et al.,* 2020). Lack of proof reading mechanism of the HIV RT is one major factor contributing to the high diversity of HIV subtypes(Nduva *et al.,* 2020; Santoro & Perno, 2013).

In Kenya and other East African countries, HIV subtype A1 has been shown to be most predominant circulating subtype, with subtype D and CRF A1_D (Giovanetti *et al.,* 2020). The results of this study on genetic diversity therefore agree with those reported in several other studies. Genetic variations ranging between 15-20% have been reported within the same HIV subtypes whereas genetic variations of 25-35% have been reported between different HIV subtypes (Nduva *et al.,* 2020). Similarly, several sub-clusters were observed in the HIV subtype A1 cluster pointing out to the high diversity of HIV even within a particular subtype in this population as previously reported and presence of intra-subtype clusters as previously demonstrated (Désiré *et al.,* 2018). Subtype A1 which was the most predominant subtype in the population exhibited 12 different sub-clusters on the phylogram tree. Subtype A1 and A2 clustered close together in the phylogenetic tree indicating a similar evolutionary trend between the two subtypes.

Subtype A2 clustered next to subtype C although the two did not seem to take the same evolutionary route. Subtype C seemed to have a closer evolutionary path with subtype G compared to other subtypes. In the present study, subtype A1 was found to be more highly divergent than subtypes D and C in Kenya as previously reported (Désiré *et al.,* 2018; Nduva *et al.,* 2020). Majority of the CRFs (79%) contained subtype A1 agreeing with other studies that have reported presence of subtype A1 in majority of the characterized CRFs (Nduva *et al.,* 2020).

5.2 Conclusions

From the forgoing, it can be concluded that:

1. HIV viral RNA quantification (viral load testing) and drug resistance testing may be an important predictor of treatment in HIV seropositive patients receiving ART in this population with 62.1% of those with virologic failure having one or more DRAMs that conferred HIVDR to one or more of the available classes of drugs. However, a combination of HIV viral load testing and drug resistance testing could improve the treatment failure prediction outcomes by eliminating patients whose treatment failure is not associated with DRAMs and resistance to anti-HIV medications but may be associated with adherence issues.
2. Several DRAMs conferring resistance to different classes of drugs were present in the study population, with M184V/I being the most prevalent NRTI DRAM, K103N/I being the most prevalent NNRTI DRAM while M46I/K was the most prevalent PI DRAM. These DRAMs were found either in isolation or in combination with other mutations rendering phenotypic susceptibility or varying degrees of phenotypic resistance to different classes of ART drugs. Whereas some patients had phenotypic susceptibility to one, a few or all ART drugs, others had low, intermediate to high level phenotypic resistance to one or more of the ART drugs studied. A possible high level of cross resistance between NRTIs and NNRTIs was observed. Cross resistance within the first generation NNRTIs (NVP and EFV) and

within second generation NNRTI (ETR and RPV) as well as between first generation and second generation NNRTIs.

3. The most predominant subtype circulating in the population is A1, with high prevalence of HIV subtype D prevalence and A1_D. From phylogenetic analysis, inter and intra-subtype variability was demonstrated with differential clustering of sequences even within subtypes.
4. There was demonstrated inter and intra-subtype variability with differential clustering of sequences even within subtypes. The HIV epidemic in Kenya is fueled from different geographic locations. Subtype variability seemed to play a part in development of both genotypic and phenotypic drug resistance. There is an increased subtype D prevalence in the population, indicating the possibility of cross-border infections.

5.3 Limitations of the study

1. There were no baseline resistance profiles for all the participants involved in this study. It was therefore not possible to determine whether or not the mutations found in this population were inherited or were as a result of drug pressure. All DRAMs found in this study were therefore assumed to be acquired
2. The study depended on self-reported drug adherence which could have been misleading.

5.4 Recommendations

1. I recommend the inclusion of drug resistance testing in the routine monitoring of treatment failure. Combination of viral load and drug resistance testing would be best for determining treatment failure and assisting in making a decision on regimen change. Since not every treatment failure can be associated with DRAMs, drug resistance testing should be provided to rule out adherence related treatment failure before any regimen switch is effected

2. I recommend provision of DRT to rule out adherence related treatment failure before any regimen switch is effected. There is need for more studies to clearly define the prevalence of ETR and RPV related mutations in populations.
3. I recommend continued monitoring of circulating HIV-1 subtypes in the general population as treatment options become increasingly available in resource-poor settings. This will eventually help in understanding the dynamics of HIV strains locally and in the region for better management of HIV infected people. I recommend continued monitoring of the circulating subtypes, especially with the reported increase in CRFs to help predict treatment failure patterns, transmission rates, rates of recombination and disease progression within populations.
4. I recommend continued monitoring of the phylogenetic relations between circulating HIV subtypes for better understanding on the entry points through which the Kenyan epidemic is fueled.

REFERENCES

Adungo, F. O., Gicheru, M. M., Adungo, N. I., Matilu, M. M., Lihana, R. W., & Khamadi, S. A. (2014). Diversity of Human Immunodeficiency Virus Type-1 Subtypes in Western Kenya. *World Journal of AIDS*, *04*(04), 365–372.

AIDSinfo. (2017). Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection Adherence to Antiretroviral Therapy in Children and Adolescents Living with HIV. AIDS Info. Retrived from https://clinicalinfo.hiv.gov/en/guidelines/pediatric-arv/adherence-antiretroviral-therapy-children-and-adolescents-living-hiv.

Anstett, K., Brenner, B., Mesplede, T., & Wainberg, M. A. (2017). HIV drug resistance against strand transfer integrase inhibitors. *Retrovirology*. *14*(1), 36 - 7.

Bain, L. E., Nkoke, C., & Noubiap, J. J. N. (2017). UNAIDS 90–90–90 targets to end the AIDS epidemic by 2020 are not realistic: Comment on "Can the UNAIDS 90–90–90 target be achieved? A systematic analysis of national HIV treatment cascades." *BMJ Global Health*. *2*(2), e000227.

Bbosa, N., Kaleebu, P., & Ssemwanga, D. (2019). HIV subtype diversity worldwide. *Current Opinion in HIV and AIDS*, *14*(3), 153–160.

Bessong, P. O., Matume, N. D., & Tebit, D. M. (2021). Potential challenges to sustained viral load suppression in the HIV treatment programme in South Africa: a narrative overview. *AIDS Research and Therapy*, *18*(1), 1–17.

Bissio, E., Barbás, M. G., Kademián, S., Bouzas, M. B., Salomón, H., Cudolá, A., … Falistocco, C. (2017). Prevalence of rilpivirine resistance in people starting antiretroviral treatment in Argentina. *Antiviral Therapy*, *22*(7), 625–629.

Brijkumar, J., Johnson, B. A., Zhao, Y., Edwards, J., Moodley, P., Pathan, K., Pillay, S.,

… Marconi, V. C. (2020). A packaged intervention to improve viral load monitoring within a deeply rural health district of South Africa. *BMC Infectious Diseases*. *20*, 836. https://doi.org/10.1186/s12879-020-05576-5.

Budambula, V., Musumba, F. O., Webale, M. K., Kahiga, T. M., Ongecha-Owuor, F., Kiarie, J. N., Sowayi, G. A., … & Were, T. (2015). HIV-1 protease inhibitor drug resistance in Kenyan antiretroviral treatment-naive and -experienced injection drug users and non-drug users. *AIDS Research and Therapy*, *12*(1), 27.

Bulage, L., Ssewanyana, I., Nankabirwa, V., Nsubuga, F., Kihembo, C., Pande, G., Ario, A. R., … & Kiyaga, C. (2017). Factors Associated with Virological Non-suppression among HIV-Positive Patients on Antiretroviral Therapy in Uganda, August 2014-July 2015. *BMC Infectious Diseases*. *17*(1), 326.

Bulstra, C. A., Hontelez, J. A. C., Otto, M., Stepanova, A., Lamontagne, E., Yakusik, A., El-Sadr, W. M., … & Vaughan, J. (2021). Integrating HIV services and other health services: A systematic review and metaanalysis. *PLoS Medicine*. *18*(11). https://doi.org/10.1371/journal.pmed.1003836

Chadha, V. K. (2006). Sample size determination in health studies. *NTI Bullettin*, 55–62.

Cherutich, P., Kim, A. A., Kellogg, T. A., Sherr, K., Waruru, A., De Cock, K. M., & Rutherford, G. W. (2016). Detectable HIV Viral Load in Kenya: Data from a Population-Based Survey. *PLOS ONE*, *11*(5), e0154318.

Cheung, K. W., Peng, Q., He, L., Cai, K., Jiang, Q., Zhou, B., To, S. W. C., …&Wang, H. (2016). Rapid and simultaneous detection of major drug resistance mutations in reverse transcriptase gene for HIV-1 CRF01-AE, CRF07-BC and subtype B in China using sequenom MassARRAY® system. *PLoS ONE*, *11*(4), e0153641.

Ciuffi, A. (2016). The benefits of integration. *Clinical Microbiology and Infection*, *22*(4), 324–332. https://doi.org/10.1016/j.cmi.2016.02.013

Clutter, D. S., Jordan, M. R., Bertagnolio, S., & Shafer, R. W. (2016). HIV-1 drug resistance and resistance testing. *Infection, Genetics and Evolution*. *46*, 292–307.

Coffin, J. M., Bale, M. J., Wells, D., Guo, S., Luke, B., Zerbato, J. M., Sobolewski, M. D., … & Hughes, S. H. (2021). Integration in oncogenes plays only a minor role in determining the in vivo distribution of HIV integration sites before or during suppressive antiretroviral therapy. *PLoS Pathogens*, *17*(4), 1–28.

Cohen, M. S., Chen, Y. Q., McCauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., Hakim, J. G., … & Fleming, T. R. (2016). Antiretroviral Therapy for the Prevention of HIV-1 Transmission. *New England Journal of Medicine*. *375*(9), 830–839.

de Cock, K. M., Jaffe, H. W., & Curran, J. W. (2021). Reflections on 40 years of AIDS. *Emerging Infectious Diseases*. *27*(6), 1553–1560.

Deeks, S. G., Overbaugh, J., Phillips, A., & Buchbinder, S. (2015). HIV infection. *Nature Reviews Disease Primers*. *1*, 15035.

del Rio, C. (2017). The global HIV epidemic: What the pathologist needs to know. *Seminars in Diagnostic Pathology*. *34*(4), 314–317.

Derache, A., Wallis, C. L., Vardhanabhuti, S., Bartlett, J., Kumarasamy, N., & Katzenstein, D. (2016). Phenotype, genotype, and drug resistance in Subtype C HIV-1 infection. *Journal of Infectious Diseases*, *213*(2), 250–256.

Désiré, N., Cerutti, L., Le Hingrat, Q., Perrier, M., Emler, S., Calvez, V., … & Visseaux, B. (2018). Characterization update of HIV-1 M subtypes diversity and

proposal for subtypes A and D sub-subtypes reclassification. *Retrovirology*, *15*(1). https://doi.org/10.1186/s12977-018-0461-y.

Diphoko, T., Gaseitsiwe, S., Kasvosve, I., Moyo, S., Okatch, H., Musonda, R., … & Essex, M. (2018). Prevalence of Rilpivirine and Etravirine Resistance Mutations in HIV-1 Subtype C-Infected Patients Failing Nevirapine or Efavirenz-Based Combination Antiretroviral Therapy in Botswana. *AIDS Research and Human Retroviruses*. *34*(8), 667–671.

Dube, MP. , & Stein JH., A. J. (2018). Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. *Department of Health and Human Services*, *40*(Build 29393). Retrieved from https://aidsinfo.nih.gov/contentfiles/lvguidelines/ adultandadolescentgl.pdf.

Dutta, N., & Saha, M. K. (2018). Virologic failure on anti-retroviral therapy without HIV drug resistance mutation Application of Nanomaterials in increased functionalization of macromolecules. *Journal of Immune Disorders. 1*(1).

Engelman, A., & Cherepanov, P. (2012). The structural biology of HIV-1: Mechanistic and therapeutic insights. In *Nature Reviews Microbiology 10*(4) 279–290.

Faria, N. R., Vidal, N., Lourenco, J., Raghwani, J., Sigaloff, K. C. E., Tatem, A. J., Van De Vijver, D. A. M., … & Dellicour, S. (2019). Distinct rates and patterns of spread of the major HIV-1 subtypes in Central and East Africa. *PLoS Pathogens*. *15*(12), e1007976.

Ferrand, R. A. (2017). Gaps in the Early Infant Diagnosis cascade in a high HIV prevalence setting. *Public Health Action*. *7*(2),78.

Freed, E. O. (2015). HIV-1 assembly, release and maturation. *Nature Reviews Microbiology*. *13*(8), 484–496.

Gaitho, D., Kinoti, F., Mwaniki, L., Kemunto, D., Ogoti, V., Njigua, C., Kubo, E., …& Mecha, J. (2021). Factors associated with the timely uptake of initial HIV virologic test among HIV-exposed infants attending clinics within a faith-based HIV program in Kenya; a cross-sectional study. *BMC Public Health*. *21*(1), 569.

Ganapathy, D., & Mozhi, K. (2021). Awareness of management of children with hiv among dental students. *International Journal of Dentistry and Oral Science*. *08*(03), 2099-2103.

Gartner, M. J., Roche, M., Churchill, M. J., Gorry, P. R., & Flynn, J. K. (2020). Understanding the mechanisms driving the spread of subtype C HIV-1. *EBioMedicine, 53,* 102682.

Gibson, R. M., Nickel, G., Crawford, M., Kyeyune, F., Venner, C., Nankya, I., Nabulime, E., … & Arts, E. J. (2017). Sensitive detection of HIV-1 resistance to Zidovudine and impact on treatment outcomes in low- to middle-income countries. *Infectious Diseases of Poverty*, *6*(1), 017-0377-0.

Gill, M. M., Hoffman, H. J., Ndatimana, D., Mugwaneza, P., Guay, L., Ndayisaba, G. F., Bobrow, E. A., … & Mofenson, L. M. (2017). 24-month HIV-free survival among infants born to HIV-positive women enrolled in Option B+ program in Kigali, Rwanda: The Kabeho Study. *Medicine (United States)*. *96*(51), e9445.

Giovanetti, M., Ciccozzi, M., Parolin, C., & Borsetti, A. (2020). Molecular epidemiology of hiv-1 in african countries: A comprehensive overview. *Pathogens*, *9*(12), 1–10.

Gomes, S. T. M., Da Silva G. E., Gomes, É. R., Queiroz, M. A. F., Júnior, E. C. S., De Vasconcelos, M. J. M., Da Silva Lemos, P., … & Vallinoto, A. C. R. (2020). Immune escape mutations in HIV-1 controllers in the Brazilian Amazon

region. *BMC Infectious Diseases*, *20*(1), 1–11.

Gounder, K., Oyaro, M., Padayachi, N., Zulu, T. M., De Oliveira, T., Wylie, J., Ndung'u, T., … & Ndung'u, T. (2017). Complex Subtype Diversity of HIV-1 Among Drug Users in Major Kenyan Cities | AIDS Research and Human Retroviruses. *AIDS Research and Human Retroviruses*. *33*(5), 500–510.

Govender, R. D., Hashim, M. J., Khan, M. A., Mustafa, H., & Khan, G. (2021). Global Epidemiology of HIV/AIDS: A Resurgence in North America and Europe. *Journal of Epidemiology and Global Health*, *11*(3), 296–301.

Günthard, H. F., Saag, M. S., Benson, C. A., Del Rio, C., Eron, J. J., Gallant, J. E., Hoy, J. F., … & Volberding, P. A. (2016). Antiretroviral drugs for treatment and prevention of HIV infection in Adults: 2016 recommendations of the international antiviral society-USA Panel. *JAMA - Journal of the American Medical Association*, *316*(2), 191–210.

Häggblom, A., Santacatterina, M., Neogi, U., Gisslen, M., Hejdeman, B., Flamholc, L., & Sönnerborg, A. (2017). Effect of therapy switch on time to second-line antiretroviral treatment failure in HIV-infected patients. *PLoS ONE*, *12*(7), e0180140.

Hamers, R. L., Rinke de Wit, T. F., & Holmes, C. B. (2018). HIV drug resistance in low-income and middle-income countries. *The Lancet HIV*. *5*(10), e588–e596.

Hassan, A. S., Mwaringa, S. M., Obonyo, C. A., Nabwera, H. M., Sanders, E. J., Rinke De Wit, T. F., … & Berkley, J. A. (2013). Low prevalence of transmitted HIV type 1 drug resistance among antiretroviral-naive adults in a rural HIV clinic in Kenya. *AIDS Research and Human Retroviruses*, *29*(1), 129–135.

Holec, A. D., Mandal, S., Prathipati, P. K., & Destache, C. J. (2017). Nucleotide Reverse

Transcriptase Inhibitors: A Thorough Review, Present Status and Future Perspective as HIV Therapeutics. *Current HIV Research*. *15*(6), 411–421.

Huarte, N., Carravilla, P., Cruz, A., Lorizate, M., Nieto-Garai, J. A., Kräusslich, H. G., Pérez-Gil, J., … & Nieva, J. L. (2016). Functional organization of the HIV lipid envelope. *Scientific Reports, 6*, 34190.

Hung, M., Tokarsky, E. J., Lagpacan, L., Zhang, L., Suo, Z., & Lansdon, E. B. (2019). Elucidating molecular interactions of L-nucleotides with HIV-1 reverse transcriptase and mechanism of M184V-caused drug resistance. *Communications Biology*, 2, 469.

Ibe, S., & Sugiura, W. (2011). Clinical significance of HIV reverse-transcriptase inhibitor-resistance mutations. *Future Microbiology*, *6*(3), 295–315. https://doi.org/10.2217/fmb.11.7.

Inzaule, S., Weidle, P. J., Yang, C., Ndiege, K., Hamers, R. L., Rinke de Wit, T. F., Thomas, T., … & Zeh, C. (2016). Prevalence and dynamics of the K65R drug resistance mutation in HIV-1-infected infants exposed to maternal therapy with lamivudine, zidovudine and either nevirapine or nelfinavir in breast milk. *The Journal of antimicrobial chemotherapy*. *71*(6), 1619–1626.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2016). Global AIDS Update 2016. *World Health Organization*. Retrieved from https://doi.org/ISBN 978-92-9253-062-5.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2018a). *90-90-90: treatment for all, UNAIDS*. Miles To Go - Unaids Data 2018. Retrieved from https://www.unaids.org/en/resources/909090.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2018b). *Global HIV & AIDS statistics — 2018 fact sheet, UNAIDS*. UNAIDS. Retrieved from

https://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2018c). *KENYA, 2018*. Country Factsheets. Retrieved from https://www.unaids.org/en/ regionscountries/ countries/kenya.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2018d). *UNAIDS Data 2018*. 1–376. Retrieved from https://www.unaids.org/sites/default/files/ media_asset/unaids-data-2018_en.pdf.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2020a). Fact Sheet - Latest Global and Regional Statistics on the Status of the AIDS Epidemic. *Joint United Nations Programme on HIV/AIDS (UNAIDS)*. Retrieved from https://irp.cdn-website.com/d7ac924f/files/uploaded/UNAIDS_ FactSheet _en.pdf.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2020b). *GLOBAL FACTSHEETS,2020, HIV and AIDS Estimates Adults and children living with with HIV*. 1–2. Retrieved from https://www.kff.org/global-health-policy/fact-sheet/the-global-hivaids-epidemic/.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2021a). FACT SHEET 2021 Global HIV Statistics. *Ending the AIDS Epidemic*, 1–3. Retrieved from https://www.unaids.org/en/resources/fact-sheet.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2021b). Global HIV Statistics. *Fact Sheet 2021*, *June*, 1. Retrieved from https://www.unaids.org/ sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf.

Joint United Nations Programme on HIV/AIDS *(UNAIDS). (2011) World AIDS Day report | UNAIDS*. Retrieved from https://reliefweb.int/report/world/unaids-

world-aids-day-report-2011.

Joint United Nations Programme on HIV/AIDS (UNAIDS). (2020). Global HIV & AIDS statistics — 2020 fact sheet | UNAIDS. Retrieved from https://www.aidsdatahub.org/sites/default/files/resource/unaids-2020-global-aids-factsheets.pdf

Joram, S. L., Paul, G., Moses, K., Stanley, B., Isaac, M., Allan, G., Tom, M., … & Mildred, M. (2017). Misdiagnosis of HIV treatment failure based on clinical and immunological criteria in Eastern and Central Kenya. *BMC Infectious Diseases*, *17*(1), 1–7.

Kantor, R., DeLong, A., Balamane, M., Schreier, L., Robert M. J., InjeraLydia, W., Kamle, F. M., … & Diero, L. (2014). HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in Western Kenya. *Journal of the International AIDS Society*. *17*(1), 19262.

Kapaata, A., Lyagoba, F., Ssemwanga, D., Magambo, B., Nanyonjo, M., Levin, J., Mayanja, B. N., … & Kaleebu, P. (2013). HIV-1 subtype distribution trends and evidence of transmission clusters among incident cases in a rural clinical cohort in southwest Uganda, 2004-2010. AIDS research and human retroviruses, *29*(3), 520–527.

Kassaye, S. G., Wang, C., Ocampo, J. M. F., Wilson, T. E., Anastos, K., Cohen, M., Greenblatt, R. M., … & Plankey, M. (2019). Viremia trajectories of HIV in HIV-positivewomen in the United States, 1994-2017. *JAMA Network Open*. *2*(5):e193822.

Kemnic, T. R., & Gulick, P. G. (2019). HIV Antiretroviral Therapy. In *StatPearls*.

Kenya National Bureau of Statistics. (2013). StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Retrieved from https://www.ncbi.nlm

.nih.gov/books/NBK513308/.

Khamadi, S. A., Ochieng, W., Lihana, R. W., Kinyua, J., Muriuki, J., Mwangi, J., Lwembe, R., … & Songok, E. M. (2005). HIV type 1 subtypes in circulation in Northern Kenya. *AIDS Research and Human Retroviruses*, *21*(9), 810–814.

Kharsany A. B. M., Cawood C., Lewis L., Yende-Zuma N., Khanyile D., Puren A., Madurai S., … & Abdool Karim Q. (2019). Trends in HIV Prevention, Treatment, and Incidence in a Hyperendemic Area of KwaZulu-Natal, South Africa. *JAMA Netw Open. 2*(11), e1914378.

Kharsany, A. B. M., & Karim, Q. A. (2016). HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. *The Open AIDS Journal*, *10*(1), 34–48.

Kim, M. B., Giesler, K. E., Tahirovic, Y. A., Truax, V. M., Liotta, D. C., & Wilson, L. J. (2016). CCR5 receptor antagonists in preclinical to phase II clinical development for treatment of HIV. *Expert Opinion on Investigational Drugs*. *25*(12):1377-1392.

Kimanga, D. O., Ogola, S., Umuro, M., Ng'ang'a, A., Kimondo, L., Murithi, P., Muttunga, J., … & Kim, A. A. (2014). & KAIS Study Group (2014). Prevalence and incidence of HIV infection, trends, and risk factors among persons aged 15-64 years in Kenya: results from a nationally representative study. *Journal of acquired immune deficiency syndromes,* (2014), *66* Suppl 1(Suppl 1), S13–S26.

Koigi, P., Ngayo, M. O., Khamadi, S., Ngugi, C., & Nyamache, A. K. (2014). HIV type 1 drug resistance patterns among patients failing first and second line antiretroviral therapy in Nairobi, Kenya. *BMC Research Notes*. 7, 890.

Krauß, J., & Bracher, F. (2018). Pharmacokinetic enhancers (Boosters)—escort for drugs against degrading enzymes and beyond. *Scientia Pharmaceutica*. 2018; *86*(4), 43.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA11: Molecular Evolutionary Genetics Analysis Version 11.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874.

Kyeyune, F., Gibson, R. M., Nanky, I., Venner, C., Metha, S., Akao, J., Ndashimye, E., … & Quiñones-Mateua, M. E. (2016). Low-Frequency Drug Resistance in HIV-Infected Ugandans on Antiretroviral Treatment Is Associated with Regimen Failure. *Antimicrobial Agents and Chemotherapy*, *60*(6), 3380–3397.

Lai, M. T., Munshi, V., Lu, M., Feng, M. Z., Hrin-Solt, R., McKenna, P. M., Hazuda, D. J., & Miller, M. D. (2016). Mechanistic study of common non-nucleoside reverse transcriptase inhibitor-resistant mutations with K103N and Y181C substitutions. *8*(10), 263.

Lavu, E., Kave, E., Mosoro, E., Markby, J., Aleksic, E., Gare, J., Elsum, I. A., … & Jordan, M. R. (2017). High levels of transmitted HIV drug resistance in a study in Papua New Guinea. *PLoS ONE*. *12*(2), e0170265.

Lel, R., Ngaira, J., Lihana, R., & Khamadi, S. (2014). HIV-1 drug resistance mutations among infants born to HIV-positive mothers in Busia, Kenya. *AIDS Research and Human Retroviruses*. *30*(12), 1236–1238.

Lenjiso, G. A., Endale, B. S., & Bacha, Y. D. (2019). Clinical and immunological failure among HIV-positive adults taking first-line antiretroviral therapy in Dire Dawa, eastern Ethiopia. *BMC Public Health*. *19*(1), 771.

Levine, M., Beck, I., Styrchak, S., Pepper, G., & Frenkel, L. (2016). Comparison of

matrix-based and filter paper-based systems for transport of plasma for HIV-1 RNA quantification and amplicon preparation for genotyping. *Journal of Clinical Microbiology*. *54*(7), 1899–1901.

Lihana, R. W., Khamadi, S. A., Lwembe, R. M., Kinyua, J. G., Muriuki, J. K., Lagat, N. J., Okoth, F. A., … Songok, E. M. (2009). HIV-1 subtype and viral tropism determination for evaluating antiretroviral therapy options: An analysis of archived Kenyan blood samples. *BMC Infectious Diseases*, *9*(1), 1–8.

Macharia, G. N., Yue, L., Staller, E., Dilernia, D., Wilkins, D., Song, H., McGowan, E., … & Gilmour, J. (2020). Infection with multiple HIV-1 founder variants is associated with lower viral replicative capacity, faster cd4+ t cell decline and increased immune activation during acute infection. *PLoS Pathogens*. *16*(9), e1008853.

Makwaga, O., Adhiambo, M., Mulama, D. H., Muoma, J., Adungo, F., Wanjiku, H., Ongaya, A., … & Mwau, M. (2020). Prevalence of human immunodeficiency virus-1 drug-resistant mutations among adults on first- and second-line antiretroviral therapy in a resource-limited health facility in Busia County, Kenya. *The Pan African Medical Journal*. *37*, 311.

Marin, R. C., Behl, T., Negrut, N., & Bungau, S. (2021). Management of antiretroviral therapy with boosted protease inhibitors—darunavir/ritonavir or darunavir/cobicistat. *Biomedicines*, *9*(3), 1–35.

Ministry of Health, & National AIDS and STI Control Programme. (2016). *Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya - 2016 Edition*. Retrieved from https://www.prepwatch.org/wp-content/uploads/2016/08/Guidelines-on-ARV-for-Treating-Preventing-HIV-Infections-in-Kenya.pdf.

Mouradjian, M. T., Heil, E. L., Sueng, H., & Pandit, N. S. (2020). Virologic suppression

in patients with a documented M184V/I mutation based on the number of active agents in the antiretroviral regimen. *8*, 2050312120960570. *SAGE Open Medicine*. https://doi.org/10.1177/2050312120960570.

Moyo, S., Hunt, G., Zuma, K., Zungu, M., Marinda, E., Mabaso, M., Kana, V., … & Rehle, T. (2020). HIV drug resistance profile in South Africa: Findings and implications from the 2017 national HIV household survey. *PLoS ONE*. *15*(11), e0241071.

Mwau, M., Syeunda, C. A., Adhiambo, M., Bwana, P., Kithinji, L., Mwende, J., Oyiengo, L., … Boeke, C. E. (2018). Scale-up of Kenya's national HIV viral load program: Findings and lessons learned. *PLoS ONE*. *13*(1), e0190659.

Nabukeera, S., Kagaayi, J., Makumbi, F. E., Mugerwa, H., & Matovu, J. K. B. (2021). Factors associated with virological non-suppression among HIV-positive children receiving antiretroviral therapy at the Joint Clinical Research Centre in Lubowa, Kampala Uganda. *PLoS ONE*. *16*(1), e0246140.

Nadai, Y., Eyzaguirre, L. M., Constantine, N. T., Sill, A. M., Cleghorn, F., Blattner, W. A., & Carr, J. K. (2008). Protocol for nearly full-length sequencing of HIV-1 RNA from plasma. *PLoS ONE*. *3*(1), e1420.

National AIDS and STI Control Programme (NASCOP), Ministry of Health, Kenya. (2018). *Kenya-ARV-Guidelines-2018-Final_20thAug2018*. *217*, 6. http://cquin.icap. columbia.edu/wp-content/uploads/2017/04/ICAP_CQUIN_Kenya-ARV-Guidelines-2018-Final_20thAug2018.pdf.

National AIDS Control Council Kenya (NACC), Ministry of Health, Kenya. (2014). Kenya AIDS Response Progress Report Progress towards Zero. *Ministry of Health, Government of Kenya.*, *March*, 37. Retrieved from https://www.unaids.org/sites/default/files/country/documents/KEN_narrative_report_2014.pdf.

National AIDS Control Council Kenya (NACC). (2016). *Kenya Aids progress report 2016*. 92. *Ministry of Health, Government of Kenya.* Retrieved from https://nacc.or.ke/wp-content/uploads/2016/11/Kenya-AIDS-Progress-Report_web.pdf.

National AIDS and STI Control Programme (NASCOP). (2018). Preliminary KENPHIA 2018 Report. *NASCOP*. Retrieved from https://doi.org/10.20961/ge.v4i1.19180.

National AIDS Control Council. (2018). Kenya HIV Estimates Report 2018. *Kenya HIV Estimates*. Retrieved from https://doi.org/10.1111/j.1365-2664.2007.0.

Nduva, G. M., Hassan, A. S., Nazziwa, J., Graham, S. M., Esbjörnsson, J., & Sanders, E. J. (2020). HIV-1 Transmission Patterns Within and Between Risk Groups in Coastal Kenya. *Scientific Reports*. *10*(1), 6775.

Ng'uni, T., Chasara, C., & Ndhlovu, Z. M. (2020). Major Scientific Hurdles in HIV Vaccine Development: Historical Perspective and Future Directions. *Frontiers in Immunology*. *11*, 590780.

Office of AIDS Research Advisory Council. (2019). Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States. Retrieved from *https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/Perinatal_GL.pdf.*

Okal, J., Lango, D., Matheka, J., Obare, F., Ngunu-Gituathi, C., Mugambi, M., & Sarna, A. (2020). "It is always better for a man to know his HIV status" - A qualitative study exploring the context, barriers and facilitators of HIV testing among men in Nairobi, Kenya. *PLoS ONE*. *15*(4), e0231645.

Onyango, D. O., van der Sande, M. A. B., Musingila, P., Kinywa, E., Opollo, V., Oyaro,

B., Nyakeriga, E., … & Rutherford, G. W. (2021). High HIV prevalence among decedents received by two high-volume mortuaries in Kisumu, Western Kenya, 2019. *PLoS ONE*, *16*(7), 1–14.

Oyaro, M., Mbithi, J., Oyugi, F., Laten, A., Anzala, O., & Engelbrecht, S. (2011). Molecular characterization of HIV type 1 among HIV-infected respondents in a cohort being prepared for HIV phase III vaccine clinical trials, Western Kenya. *AIDS Research and Human Retroviruses*. *27*(3), 257–264.

Patel, R. C., Oyaro, P., Odeny, B., Mukui, I., Thomas, K. K., Sharma, M., Wagude, J., … & Abuogi, L. L. (2020). Optimizing viral load suppression in Kenyan children on antiretroviral therapy (Opt4Kids). *Contemporary Clinical Trials Communications*. *20*, 100673.

Phillips, A. N., Stover, J., Cambiano, V., Nakagawa, F., Jordan, M. R., Pillay, D., … & Bertagnolio, S. (2017). Impact of HIV drug resistance on HIV/AIDS-associated mortality, new infections, and antiretroviral therapy program costs in Sub-Saharan Africa. *Journal of Infectious Diseases*, *215*(9), 1362–1365.

QIAGEN. (2012). RNeasy Mini RNA extraction. *Manual*. Retrieved from https://doi.org/10.1007/978-0-387-77674-3.

Recordon-Pinson, P., Alves, B. M., Tumiotto, C., Bellecave, P., Bonnet, F., Neau, D., … & Fleury, H. (2018). A New HIV-1 Circulating Recombinant Form (CRF98-cpx) Between CRF06-cpx and Subtype B Identified in Southwestern France. *AIDS Research and Human Retroviruses*. *34*(11), 1005–1009.

Reid-Bayliss, K. S., & Loeb, L. A. (2017). Accurate RNA consensus sequencing for high-fidelity detection of transcriptional mutagenesis-induced epimutations. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(35), 9415–9420.

Reis, M. N. G., Guimarães, M. L., Bello, G., & Stefani, M. M. A. (2019). Identification of new HIV-1 circulating recombinant forms CRF81-cpx and CRF99-BF1 in central Western Brazil and of unique BF1 recombinant forms. *Frontiers in Microbiology*. *10*, 97.

Richetta, C., Thierry, S., Thierry, E., Lesbats, P., Lapaillerie, D., Munir, S., … Delelis, O. (2019). Two-long terminal repeat (LTR) DNA circles are a substrate for HIV-1 integrase. *Journal of Biological Chemistry*, *294*(20), 8286–8295.

Rodger, A. J., Cambiano, V., Bruun, T., Vernazza, P., Collins, S., Van Lunzen, J., Corbelli, G. M., E. … & Lundgren, J. (2016). Sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy. *JAMA - Journal of the American Medical Association*, *316*(2), 171–181.

Rubio-Garrido, M., González-Alba, J. M., Reina, G., Ndarabu, A., Barquín, D., Carlos, S., … Holguín, Á. (2020). Current and historic HIV-1 molecular epidemiology in paediatric and adult population from Kinshasa in the Democratic Republic of Congo. *Scientific Reports*. *10*(1), 18461.

Ryscavage, P., Kelly, S., Li, J. Z., Richard H. P., & Taiwo, B. (2014). Significance and clinical management of persistent low-level viremia and very-low-level viremia in HIV-1-infected patients. *Antimicrobial Agents and Chemotherapy*. *58*(7), 3585-3598.

Sandbulte, M., Brown, M., Wexler, C., Maloba, M., Gautney, B., Goggin, K., … & Finocchario-Kessler, S. (2020). Maternal viral load monitoring: Coverage and clinical action at 4 Kenyan hospitals. *PLoS ONE*. *15*(5), e0232358.

Santoro, M. M., & Perno, C. F. (2013). HIV-1 Genetic Variability and Clinical Implications. *ISRN Microbiology*. *2013,* 481314

Saravanan, S., Kausalya, B., Gomathi, S., Sivamalar, S., Pachamuthu, B., Selvamuthu, P., … & Kantor, R. (2017). Etravirine and Rilpivirine Drug Resistance among HIV-1 Subtype C Infected Children Failing Non-Nucleoside Reverse Transcriptase Inhibitor-Based Regimens in South India. *AIDS Research and Human Retroviruses*. *33*(6), 567–574.

Seitz, R. (2016). Human Immunodeficiency Virus (HIV). *Transfusion Medicine and Hemotherapy*. *43*(3), 203-222..

Sharp, P. M., & Hahn, B. H. (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harbor Perspectives in Medicine*. *1*(1), a006841.

Siedner, M. J., Moorhouse, M. A., Simmons, B., de Oliveira, T., Lessells, R., Giandhari, J., … & Gupta, R. K. (2020). Reduced efficacy of HIV-1 integrase inhibitors in patients with drug resistance mutations in reverse transcriptase. *Nature Communications*. *11*(1), 5922.

Silverman, R. A., Beck, I. A., Kiptinness, C., Levine, M., Milne, R., McGrath, C. J., … & Chung, M. H. (2017). Prevalence of Pre-antiretroviral-Treatment Drug Resistance by Gender, Age, and Other Factors in HIV-Infected Individuals Initiating Therapy in Kenya, 2013-2014. *Journal of Infectious Diseases*. *216*(12), 1569–1578.

Skhosana, L., Steegen, K., Bronze, M., Lukhwareni, A., Letsoalo, E., Papathanasopoulos, M. A., … & Stevens, W. S. (2015). High prevalence of the K65R mutation in hiv-1 subtype C infected patients failing tenofovir-based first-line regimens in South Africa. *PLoS ONE*. *10*(2), e0118145.

Sluis-Cremer, N. (2014). The emerging profile of cross-resistance among the nonnucleoside HIV-1 reverse transcriptase inhibitors. *Viruses*. *6*(8), 2960–2973.

Smith, L. K., Babcock, I. W., Minamide, L. S., Shaw, A. E., Bamburg, J. R., & Kuhn, T. B. (2021). Direct interaction of HIV *gp*120 with neuronal CXCR4 and CCR5 receptors induces cofilin-actin rod pathology via a cellular prion protein- And NOX-dependent mechanism. *PLoS ONE*. *16*(3), e0248309.

Sonali, P., Santosh, W., Bhagyashri, G., Pranit, K., & Yash, J. (2019). Anti-HIV/AIDS Drugs: An Overview Pawar. *Journal of Drug Delivery & Therapeutics*. 2019 *9*(3), v9i3.2645.

Takou, D., Fokam, J., Teto, G., Santoro, M. M., Ceccherini-Silberstein, F., Nanfack, A. J., … & Ndjolo, A. (2019). HIV-1 drug resistance testing is essential for heavily-treated patients switching from first- to second-line regimens in resource-limited settings: Evidence from routine clinical practice in Cameroon. *BMC Infectious Diseases*, *19*(1), 1–9.

Teasdale, C. A., Abrams, E. J., Yuengling, K. A., Lamb, M. R., Wang, C., Vitale, M., … & El-Sadr, W. M. (2020). Expansion and scale-up of HIV care and treatment services in four countries over ten years. *PLoS ONE*, *15*(4), 1–14.

Tebit, D. M., Patel, H., Ratcliff, A., Alessandri, E., Liu, J., Carpenter, C., … & Arts, E. J. (2016). HIV-1 Group O Genotypes and Phenotypes: Relationship to Fitness and Susceptibility to Antiretroviral Drugs. *AIDS Research and Human Retroviruses*. https://doi.org/10.1089/aid.2015.0318.

Teeranaipong, P., Sirivichayakul, S., Mekprasan, S., Ruxrungtham, K., & Putcharoen, O. (2014). Rilpivirine versus etravirine validity in NNRTI-based treatment failure in Thailand. *Journal of the International AIDS Society*. *17*(4 Suppl 3), 19740.

Tian, L., Kim, M. S., Li, H., Wang, J., & Yang, W. (2018). Structure of HIV-1 reverse transcriptase cleaving RNA in an RNA/DNA hybrid. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(3), 507–

512.

Tongo, M., Dorfman, J. R., & Martin, D. P. (2016). High Degree of HIV-1 Group M (HIV-1M) Genetic Diversity within Circulating Recombinant Forms: Insight into the Early Events of HIV-1M Evolution. *Journal of Virology*, *90*(5), 2221–2229.

Tough, R. H., & McLaren, P. J. (2019). Interaction of the host and viral genome and their influence on HIV disease. *Frontiers in Genetics*. *9*, 720.

Tseng, A., Seet, J., & Phillips, E. J. (2015). The evolution of three decades of antiretroviral therapy: Challenges, triumphs and the promise of the future. *British Journal of Clinical Pharmacology*. *79*(2), 182-94.

Tuaillon, E., Kania, D., Pisoni, A., Bollore, K., Taieb, F., Ontsira N. E. N., … & Van de Perre, P. (2020). Dried Blood Spot Tests for the Diagnosis and Therapeutic Monitoring of HIV and Viral Hepatitis B and C. *Frontiers in Microbiology*, *11*(3), 1–7.

Vaillant, A. A. J., & Gulick., P. G. (2020). HIV Disease Current Practice. *National Center for Biotechnology Information*. In *StatPearls*. StatPearls Publishing. Retrieved from https://pubmed.ncbi.nlm.nih.gov/30521281/.

Vardell, E. (2020). Global Health Observatory Data Repository. *Medical Reference Services Quarterly*. *39*(1), 67-74.

Waihenya, E., Munyao, R., Sanguli, J., Mwachari, L., & Khamadi, C. (2015). HIV-1 Drug Resistance Mutations in Patients Failing 1st Line Therapy in a Comprehensive Care Center in Nairobi, Kenya. *World Journal of AIDS*, *5*(02), 83–89.

Waller, D. G., & Sampson, A. P. (2018). Chemotherapy of infections. *Medical*

Pharmacology and Therapeutics. *5*, 707-729.

Wang, Y., Xing, H., Liao, L., Wang, Z., Su, B., Zhao, Q., … & Shao, Y. (2014a). The development of drug resistance mutations K103N Y181C and G190A in long term Nevirapine-containing antiviral therapy. *AIDS Research and Therapy*. *11*(1), 36.

Wekesa, P., McLigeyo, A., Owuor, K., Mwangi, J., Nganga, E., & Masamaro, K. (2020). Factors associated with 36-month loss to follow-up and mortality outcomes among HIV-infected adults on antiretroviral therapy in Central Kenya. *BMC Public Health*, *20*(1), 1–11.

Weng, Y., Zhang, L., Huang, J., Zhao, J., Luo, P., Bi, S., … & Li, C. (2016). A simple and cost-saving phenotypic drug susceptibility testing of HIV-1. *Scientific Reports*, *6*(9), 1–9. https://doi.org/10.1038/srep33559.

World Health Organization (WHO). (2016). Global Health Sector Strategy on HIV 2016-2021. In *World Health Organization* (Issue June 2016). Retrieved from http://apps.who.int/iris/bitstream/10665/246178/1/WHO-HIV-2016.05-eng.pdf?ua=1.

World Health Organization (WHO). (2017). *HIV/AIDS Fact sheet*. Media Centre. Retrieved from https://doi.org//entity/mediacentre/factsheets/ fs297/ en/index.html

World Health Organization (WHO). Global action plan on HIV drug resistance 2017–2021. (2017). *WHO*. Retrieved from https://www.who.int/publications /i/item/978-92-4-151284-8.

Wilhelmson, S., Månsson, F., Lindman, J. L., Biai, A., Esbjörnsson, J., Norrgren, H., … & Medstrand, P. (2018). Prevalence of HIV-1 pretreatment drug resistance among treatment naïve pregnant women in Bissau, Guinea Bissau. *PLoS*

ONE, *13*(10). https://doi.org/10.1371/journal.pone.0206406.

Woodham, A. W., Skeate, J. G., Sanna, A. M., Taylor, J. R., Da Silva, D. M., Cannon, P. M., & Martin Kast, W. (2016). Human Immunodeficiency Virus Immune Cell Receptors, Coreceptors, and Cofactors: Implications for Prevention and Treatment. *AIDS Patient Care and STDs*. *30*(7), 291–306.

World Health Organization (WHO). (2016). Global Health Sector Strategy on HIV 2016-2021. *World Health Organization* (Issue June 2016). Retrieved from http://apps.who.int/iris/bitstream/10665/246178/1/WHO-HIV-2016.05-eng.pdf?ua=1.

World Health Organization (WHO). (2017). *HIV/AIDS Fact sheet*. Media Centre. Retrieved from https://doi.org//entity/mediacentre/factsheets /fs297/ en/index.html

World Health Organization (WHO). (2017). Global action plan on HIV drug resistance 2017–2021.. *WHO Press*. Retrieved from https://www.who.int/publications/ i/item/978-92-4-151284-8.

World Health Organisation. (2019). Policy Brief: Update of Recommendations on First- and Second-Line Antiretroviral Regimens. *WHO Library Cataloguing-in-Publication Data*, *July*. Retrieved from https://www.who.int/hiv/pub/arv/arv-update-2019-policy/en/.

Zhou, Y., Lu, J., Wang, J., Yan, H., Li, J., Xu, X., … & Hu, H. (2016). Prevalence of HIV Antiretroviral Drug Resistance and Its Impacts on HIV-1 Virological Failures in Jiangsu, China: A Cross-Sectional Study. *BioMed Research International*. *2016*, 1752437.

Zulfiqar, H. F., Javed, A., Sumbal, A. B., Ali, Q., Akbar, K., Nadeem, T., … & Husnain, T. (2017). HIV Diagnosis and Treatment through Advanced Technologies.

Frontiers in Public Health. *7*(5), 32.

Zuo, L., Liu, K., Liu, H., Hu, Y., Zhang, Z., Qin, J., … & Zhang, C. (2020). Trend of HIV-1 drug resistance in China: A systematic review and meta-analysis of data accumulated over 17 years (2001–2017). *EClinicalMedicine*. *18*, 100238.

APPENDICES

Appendix I: Stanford University HIV Drug Resistance Database Generated Clinical Report

Sequence summary

SDRMs Pretty pairwise

Sequence includes PR:

codons 4 - 99

Sequence includes RT:

codons 38 - 247

Subtype:

D (5.56%)

Sequence quality assessment

PR

10203040506070809049

RT

5065809511012514015517018520021523038247

Drug resistance interpretation: PR

HIVDB 9.0 (2021-02-22)

PI Major Resistance Mutations:

None

PI Accessory Resistance Mutations:

None

Other Mutations:

I13V, K20R, E35D, R41K, K55R, L63P, I64V, I72V, V77I

Protease Inhibitors	
atazanavir/r (ATV/r)	Susceptible
darunavir/r (DRV/r)	Susceptible
fosamprenavir/r (FPV/r)	Susceptible
indinavir/r (IDV/r)	Susceptible
lopinavir/r (LPV/r)	Susceptible
nelfinavir (NFV)	Susceptible
saquinavir/r (SQV/r)	Susceptible
tipranavir/r (TPV/r)	Susceptible

PR comments

Other

- **K20R** is a highly polymorphic PI-selected accessory mutation.

Mutation scoring: PR

HIVDB 9.0 (2021-02-22)

PI	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r

PI		DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
Total	0	0	0	0	0	0	0	0

Drug resistance interpretation: RT

HIVDB 9.0 (2021-02-22)

NRTI Resistance Mutations:

K70R, **M184V**

NNRTI Resistance Mutations:

Y181C, **H221Y**

Other Mutations:

K49R, V60I, T69N, K122E, I132V, K166R, D177E, I178M, Q207E, R211K, V245K

Nucleoside Reverse Transcriptase Inhibitors

abacavir (ABC)	Low-Level Resistance
zidovudine (AZT)	Low-Level Resistance
stavudine (D4T)	Susceptible
didanosine (DDI)	Low-Level Resistance
emtricitabine (FTC)	High-Level Resistance
lamivudine (3TC)	High-Level Resistance
tenofovir (TDF)	Susceptible

Non-nucleoside Reverse Transcriptase Inhibitors

doravirine (DOR)	Intermediate Resistance
efavirenz (EFV)	Intermediate Resistance
etravirine (ETR)	Intermediate Resistance

nevirapine (NVP) High-Level Resistance

rilpivirine (RPV) High-Level Resistance

RT comments

NRTI

- **K70R** causes intermediate resistance to AZT and possibly low-level resistance to D4T, DDI, ABC and TDF.
- **M184V/I** cause high-level in vitro resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, **M184V/I** are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication.

NNRTI

- **Y181C** is a non-polymorphic mutation selected in patients receiving NVP, ETR and RPV. It reduces susceptibility to NVP, ETR, RPV, and EFV by >50-fold, 5-fold, 3-fold, and 2-fold, respectively. Although **Y181C** itself reduces EFV susceptibility by only 2-fold, it has been associated with a reduced response to an EFV-containing regimen in NNRTI-experienced patients. **Y181C** has a weight of 2.5 in the Tibotec ETR genotypic susceptibility score. Alone, it does not appear to reduce DOR susceptibility.
- **H221Y** is a non-polymorphic accessory mutation selected primarily by NVP, RPV, and DOR. It frequently occurs in combination with Y181C.

Other

- **T69N** is a relatively non-polymorphic mutation weakly selected in patients receiving NRTIs. In combination with TAMs, it may contribute minimally reduced susceptibility to ddI, d4T, and AZT.

Mutation scoring: RT

HIVDB 9.0 (2021-02-22)

NRTI	ABC	AZT	D4T	DDI	FTC	3TC	TDF
K70R	5	30	15	10	0	0	5
M184V	15	-10	-10	10	60	60	-10
Total	20	20	5	20	60	60	-5

NNRTI	DOR	EFV	ETR	NVP	RPV
Y181C	10	30	30	60	45
Y181C + H221Y	10	0	0	0	10
H221Y	15	10	10	15	15
Total	35	40	40	75	70

Appendix II: GenBank Accession Numbers

Sequence Name	GenBank Sequence ID	Sequence Name	GenBank Sequence ID
SEQ001	MW618176	SEQ034	MW618209
SEQ002	MW618177	SEQ035	MW618210
SEQ003	MW618178	SEQ036	MW618211
SEQ004	MW618179	SEQ037	MW618212
SEQ005	MW618180	SEQ038	MW618213
SEQ006	MW618181	SEQ039	MW618214
SEQ007	MW618182	SEQ040	MW618215
SEQ008	MW618183	SEQ041	MW618216
SEQ009	MW618184	SEQ042	MW618217
SEQ010	MW618185	SEQ043	MW618218
SEQ011	MW618186	SEQ044	MW618219
SEQ012	MW618187	SEQ045	MW618220

SEQ013	MW618188	SEQ046	MW618221
SEQ014	MW618189	SEQ047	MW618222
SEQ015	MW618190	SEQ048	MW618223
SEQ016	MW618191	SEQ049	MW618224
SEQ017	MW618192	SEQ050	MW618225
SEQ018	MW618193	SEQ051	MW618226
SEQ019	MW618194	SEQ052	MW618227
SEQ020	MW618195	SEQ053	MW618228
SEQ021	MW618196	SEQ054	MW618229
SEQ022	MW618197	SEQ055	MW618230
SEQ023	MW618198	SEQ056	MW618231
SEQ024	MW618199	SEQ057	MW618232
SEQ025	MW618200	SEQ058	MW618233
SEQ026	MW618201	SEQ059	MW618234

SEQ027	MW618202	SEQ060	MW618235
SEQ028	MW618203	SEQ061	MW618236
SEQ029	MW618204	SEQ062	MW618237
SEQ030	MW618205	SEQ063	MW618238
SEQ031	MW618206	SEQ064	MW618239
SEQ032	MW618207	SEQ065	MW618240
SEQ033	MW618208	SEQ066	MW618241
Sequence Name	GenBank Sequence ID	Sequence Name	GenBank Sequence ID
SEQ067	MW618242	SEQ100	MW618275
SEQ068	MW618243	SEQ101	MW618276
SEQ069	MW618244	SEQ102	MW618277
SEQ070	MW618245	SEQ103	MW618278
SEQ071	MW618246	SEQ104	MW618279
SEQ072	MW618247	SEQ105	MW618280

SEQ073	MW618248	SEQ106	MW618281
SEQ074	MW618249	SEQ107	MW618282
SEQ075	MW618250	SEQ108	MW618283
SEQ076	MW618251	SEQ109	MW618284
SEQ077	MW618252	SEQ110	MW618285
SEQ078	MW618253	SEQ111	MW618286
SEQ079	MW618254	SEQ112	MW618287
SEQ080	MW618255	SEQ113	MW618288
SEQ081	MW618256	SEQ114	MW618289
SEQ082	MW618257	SEQ115	MW618290
SEQ083	MW618258	SEQ116	MW618291
SEQ084	MW618259	SEQ117	MW618292
SEQ085	MW618260	SEQ118	MW618293
SEQ086	MW618261	SEQ119	MW618294

SEQ087	MW618262	SEQ120	MW618295
SEQ088	MW618263	SEQ121	MW618296
SEQ089	MW618264	SEQ122	MW618297
SEQ090	MW618265	SEQ123	MW618298
SEQ091	MW618266	SEQ124	MW618299
SEQ092	MW618267	SEQ125	MW618300
SEQ093	MW618268	SEQ126	MW618301
SEQ094	MW618269	SEQ127	MW618302
SEQ095	MW618270	SEQ128	MW618303
SEQ096	MW618271	SEQ129	MW618304
SEQ097	MW618272	SEQ130	MW618305
SEQ098	MW618273	SEQ131	MW618306
SEQ099	MW618274	SEQ132	MW618307

Sequence Name	GenBank Sequence ID
SEQ132	MW618307
SEQ133	MW618308
SEQ134	MW618309
SEQ135	MW618310
SEQ136	MW618311
SEQ137	MW618312
SEQ138	MW618313
SEQ139	MW618314
SEQ140	MW618315

Appendix III: Ethical Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: kemri-hq@nairobi.mimcom.net; director@kemri.org; Website: www.kemri.org

KEMRI/RES/7/3/1 **16 April 2009**

TO: **Dr. S. A. Khamadi & Mr. A. Kebira (CVR)**
(PRINCIPAL INVESTIGATORS)

THRO': **Dr. F Okoth,**
CENTRE DIRECTOR, (CVR),
NAIROBI

RE: **SSC No. 1394: EVALUATION OF VIRAL EVOLUTION AND ITS ROLE ON DEVELOPMENT OF ANTIRETROVIRAL DRUG RESISTANCE IN A COHORT STUDY.**

Dear All,

Your letter dated 8th April 2009 refers.

It is now clear that, in response to the justice issue we raised of enrolling female clients only, that you have revised the protocol to enroll both men and women. The study design has also been changed such that it will be a 5 year longitudinal study with research participants bled every 3 months.

We acknowledge receipt of the revised Informed Consent Document (ICD) and "Ethical Consideration" section study protocol and note the changes in the ICD to include the changes recommended by the Committee.

Due consideration has been given to ethical issues and the study is granted approval from today 16th April 2009 to 15th April 2010.

Please note that any changes to the research study must be reported to the Scientific Steering Committee and to the Ethical Review Committee prior to implementation. This includes changes to research design, equipment, personnel, funding or procedures that could introduce new or more than minimum risk to research participants.

Respectfully,

RCKithinji

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

In Search of Better Health

Appendix IV: Study Informed Consent Form.

Name of the study: **HIV Treatment Failure Markers; Virologic Outcomes after 12 Months of Antiretroviral Therapy among Patients Receiving HAART in Busia County, Kenya**.

Cohort Identification Number: ______________________.

Today's Date (dd / mm / yyyy): _______/________/_______.

Name/ Surname (participant): ___________________ ___________________.

Name/ Surname (interviewer): __________________ ___________________.

Hello, my name is _________________________. I am working with KEMRI NAIROBI). I am going to give you information and invite you to be in this study. Before you decide, you can ask me any questions you may have or talk to anyone else that you feel comfortable with. If I use any word that you do not understand or any part of my questions is not clear for you, please let me know and I will explain to you in more detail. If you have questions later, you can ask any KEMRI NAIROBI staff or health facility.

This study aims to evaluate whether the normal tests that are used to detect whether you have undergone HIV drug resistance are reliable. This will help your physician to know whether it is necessary to change your drugs to another group of drugs or retain you on the same group of HIV drug therapy but different combination.

Your participation and your decision to participate in this study are entirely voluntary. If you choose not to participate, all the services you receive at the health facility will continue to be offered to you. You may also choose to change your mind later and stop participating, even if you had earlier agreed to take part and changed your mind, and the services you receive at the clinic will continue to be offered to you.

The sample that will be used is the sample taken from you at the Comprehensive Care Clinic in which was collected for CD4 cell count and/viral load testing. The samples will be taken to Kenya Medical training Institute in Nairobi to test for HIV drug resistance test.

Based on your approval and the approval of the Ethical Review Committee in KEMRI the results obtained after the testing of the HIV drug resistance will be compared with the predictive tests that were used to detect the drug resistance (CD4 and/Viral load).

The results of this testing on your blood will help us determine whether the predictive tests used to detect drug resistance are reliable or another method should be in cooperated with it. This study will also assist in changing your treatment if your doctor feels that it is necessary, to an appropriate drug combination. All data obtained will be handled with confidentiality and will only be accessible to the researcher and your doctor only.

If you have any questions you may ask me now or later, even after the study has started. If you wish to ask questions later, you may call or write to the following persons:

1. James Munyao Kingoo: Principal Investigator, Telephone: 0784682788. Email: israeldominion2015@gmail.com
2. **The Kenya Medical Research Institute.** National Ethical Review Committee. P.O. Box 54840-00200.Nairobi, Kenya. Telephone: 0722205901.

Consent signing:

I have read the above information, or it has been read it to me. I have had the opportunity to ask questions about the study and I am satisfied with the information I have been given. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw the study at any time without in any way

affecting my medical care. I accept for my sample to be tested genetically for resistance to medication, I hereby do give consent for my sample to be transferred to KEMRI (tick where appropriate):

☐ **Yes:**

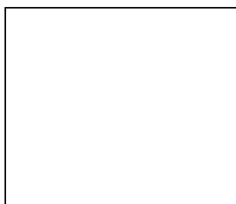
☐ **No:**

Write name of participant here: ________________________________

Today's Date (dd / mm/ yyyy): _____/_____/_______.

Signature of participant: ___________________________________.

OR



Thumbprint of participant:

Witness: (only to be used if the patient is not able to read the consent form and has given his/her thumbprint). I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I

confirm that the individual has given consent freely. I also witnessed that a copy of this Informed Consent Form has been provided to the caregiver.

Print name of witness________________________________

Signature of witness.________________________________